Red colour improvement in apple fruit (*Malus domestica* Borkh.)

By

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

Fruit red colour coverage and intensity determine the market value of bi-colour apples. Anthocyanin synthesis and therefore red colour development is highly light- and temperature dependent, the latter being especially challenging in warmer Mediterranean-type climates such as the Western Cape, South Africa. Colour development also occurs simultaneously with fruit ripening, a process that is highly ethylene sensitive. ‘Cripps’ Pink’ and older strains of ‘Fuji’ apples are notorious for poor and erratic colour development thus limiting producer profits. Bi-colour apples are therefore harvested over multiple picking dates mostly based on the extent of fruit red colour development.

Preharvest aminoethoxyvinylglycine (AVG) (125 $mg \cdot L^{-1}$), 1-aminocyclopropane-1-carboxylic acid (ACC) (200 $\mu L \cdot L^{-1}$) and a combination of the two were evaluated together with postharvest 1-methylcyclopropene (1-MCP), to determine the effect on red colour development, fruit quality and maturity of ‘Cripps’ Pink’ apples. ACC successfully increased percentage blush coverage of fruit and percentage Pink Lady™ pack-out; however, fruit maturity was advanced. AVG on its own resulted in a delay in red colour development, as well as fruit ripening. The combination of ACC and AVG resulted in fruit colour similar to that of untreated control fruit.

The effect of ACC (100 $\mu L \cdot L^{-1}$ – 400 $\mu L \cdot L^{-1}$) applied two weeks before harvest on red colour development of ‘Fuji Kiku’ (Brak) and ‘Cripps’ Pink’ apples was evaluated over one and two seasons, respectively. The internal ethylene concentration (IEC) of ‘Cripps’ Pink’ apples was determined. A rapid increase in IEC of fruit after ACC application in both seasons indicated that ACC was taken up by the fruit and converted to ethylene. This resulted in an earlier shift in harvest distribution for both cultivars, indicating stimulation of red colour development by ACC. Loss in fruit firmness, greater starch breakdown and yellower ground colour of fruit treated with higher rates of ACC (300 $\mu L \cdot L^{-1}$ and 400 $\mu L \cdot L^{-1}$) indicate that the advance in red colour development was due to advanced maturity. ACC applied at 200 $\mu L \cdot L^{-1}$ followed by postharvest 1-MCP treatment showed potential for aiding colour development of bi-colour apples without negatively affecting fruit maturity.

Two commercially available reflective mulches, viz. Lumilys® and ColorIt, were installed four to five weeks before harvest and evaluated on ‘Rosy Glow’ and ‘Fuji’ apples to improve red colour development without advancing fruit maturity. Trials were conducted in

open orchards as well as under shade netting. Both reflective fabrics performed similarly in reflecting photosynthetically active radiation and increasing red blush colour of both cultivars, especially of the lower canopy fruit, compared to fruit from trees next to grassed alleys. Fruit maturity was largely unaffected by mulch installation, but varying results in parameters such as fruit firmness of 'Rosy Glow' apples indicate that this needs to be further investigated. The high initial input cost of reflective mulches might be justified by its improvement of Class one pack-out of fruit as well as its reusability within a season and over various seasons. Overall, the application of reflective mulching seems to be the better way to improve colour in 'Cripps' Pink' and 'Fuji rather than ACC application.

OPSOMMING

ROOI KLEUR VERBETERING VAN APPELS (*MALUS DOMESTICA* BORKH.)

Die markwaarde van twee-kleurige appelkultivars word bepaal deur rooi kleur dekking en -intensiteit. Antosianiensintese, d.w.s. rooi kleurontwikkeling, is hoogs afhanklik van lig- en temperatuur en in areas met 'n warm Mediterreense-tipe klimaat soos in die Wes-Kaap, Suid Afrika is veral hoë lugtemperature dikwels problematies. Kleurontwikkeling vind ook gelyktydig plaas met vrugrypwording wat hoogs etileen sensitief is. 'Cripps' Pink' en ouer seleksies van 'Fuji' appels is bekend vir hul swak en wisselvallige rooi kleur ontwikkelling wat dus produsente se winste kan beperk. Daarom word twee-kleurige appels, na gelang van die kleurontwikkeling, oor meer as een datum ge-oes.

Die effek van aminoetoksivinielglisien (AVG) (125 $mg \cdot L^{-1}$), 1-aminosiklopropaan-1-karboksielsuur (ACC) (200 $\mu L \cdot L^{-1}$), 'n kombinasie van die twee en 'n na-oes 1-metielsiklopropeen (1-MCP) toediening is geëvalueer om hul effek op rooi kleurontwikkeling, vrugkwaliteit en -rypheid van 'Cripps' Pink' appels te bepaal. ACC het beide die persentasie blosdekking en die Pink Lady™ uitpakke verhoog, maar die vrugrypheid versnel. AVG op sy eie het rooi kleurontwikkeling en vrugrypwording vertraag. Die kombinasie van ACC an AVG het gelei tot vrugkleur soortgelyk aan dié van die onbehandelde kontrole vrugte.

Die effek van verskillende ACC dosisse (100 $\mu L \cdot L^{-1}$ – 400 $\mu L \cdot L^{-1}$), toegedien twee weke voor oes, op rooi kleurontwikkeling van 'Fuji Kiku' (Brak) en 'Cripps' Pink' appels is, onderskeidelik, oor een of twee seisoene geëvalueer. Interne etileenkonsentrasie (IEK) van 'Cripps' Pink' appels was ook gemeet. Die IEK van die vrugte het skerp toegeneem na ACC toediening in beide seisoene wat aandui dat ACC opgeneem is deur die vrugte en na etileen omgeskakel is. Die etileen het 'n vervroeging in die oes van beide kultivars veroorsaak wat daarop dui dat rypwording deur ACC gestimuleer was en rooi kleurontwikkeling daarmee saam. Hoër konsentrasies van ACC (300 $\mu L \cdot L^{-1}$ and 400 $\mu L \cdot L^{-1}$) het egter 'n skerp verlies in fermheid, hoë styselafbraak en 'n geler agtergrondkleur veroorsaak en dus die vrugrypwording versnel. 'n ACC toediening teen 200 $\mu L \cdot L^{-1}$ gevolg deur 'n na-oes 1-MCP toediening het potensiaal getoon om rooi kleurontwikkeling van twee-kleurige appels te bevorder sonder om vrugrypheid negatief te affekteer.

Twee kommersiële weerkaatsende deklae, nl. Lumilys® en ColorIt, was vier tot vyf weke voor oes geinstalleer en geëvalueer om 'Rosy Glow' en 'Fuji' se rooi kleurontwikkeling te verbeter, sonder om vrugrypheid te bevorder. Die proewe is uitgevoer in oop boorde en

boorde onder skadunet. In vergelyking met die grasry-kontrole het beide die weerkaatsende materiale beter rooi kleurontwikkeling tot gevolg gehad. Hul het soortgelyk presteer t.o.v. die weerkaatsing van fotosintetiese aktiewe lig en verbetering in rooi kleur van veral die vrugte in die onderste gedeeltes van die boom. Vrugrypheid was grotendeels nie geaffekteer deur die deklae nie, maar variërende resultate in parameters soos vrugfermheid van 'Rosy Glow' appels dui aan dat dit verder ondersoek moet word. Die hoë insetkoste van weerkaatsende deklae word moontlik geregverdig deur die verbetering in Klas-een vruguitpakke en die feit dat dit hergebruik kan word gedurende 'n seisoen en ook oor verskeie seisoene. Algeheel betrag is die gebruik van weerkaatsende deklae waarskynlik die beter roete om rooi kleur in 'Cripps' Pink' en 'Fuji' te verbeter eerder as om ACC aan te wend.

This thesis is a compilation of chapters, starting with a literature review, followed by three research papers. Each paper was prepared as a scientific paper for submission to *HortScience*. English (United States) was therefore used throughout the thesis except in the Summary. Repetition or duplication between papers might therefore be unavoidable.

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GENERAL INTRODUCTION

The South African apple industry is competitive both locally and internationally. During 2019, out of a total of 894 306 metric tons of apples (*Malus domestica Borkh.)* produced, 47% were exported and 22% were sold locally (Hortgro, 2019). Export markets are more favorable economically to producers than local markets but have strict fruit appearance and quality requirements driven by consumer preference and cultivar-specific trademarks. Fruit quality demand varies globally in terms of fruit characteristics. Consumers seem to favor fruit with a more pronounced red blush development, since this is associated with a higher antioxidant content and thus greater health benefits than fruit with less blush coverage (Csihon and Gonda, 2016; Hamadziripi et al., 2014). In recent years bi-color cultivars have become more popular.

Two examples of successful bi-color apple cultivars that are cultivated in South Africa are 'Fuji', which accounted for 9% (2 197 ha), and 'Cripps' Pink', which accounted for 12% (3 063 ha) of the total area planted to apples in South Africa in 2019 (Hortgro, 2019). 'Cripps' Pink' apples are sold under the tradename Pink Lady™, provided that they have, amongst others, a redcolored surface area of at least 60% for Asian and Gulf State territories and 40% for all other markets (Anon., 2020). Similarly, 'Fuji' apples require a red blush coverage of 50%. 'Cripps' Pink' and older strains of 'Fuji' apples are notorious for poor and erratic color development (Shafiq et al., 2011), especially in warmer Mediterranean-type climates such as in South Africa.

Red color development is a result of anthocyanin biosynthesis and accumulation, often coinciding with simultaneous degradation of chlorophyll in the fruit peel (Tijskens et al., 2011). Red color development in apple fruit is influenced by genetic, environmental, and developmental factors as well as cultural practices (Saure, 1990). Exposure of fruit to light is critical to red color development as well as optimal photosynthetic activity to sustain plant and fruit growth (Shafiq et al., 2014). Pruning strategies (Palmer et al., 1992) and installing reflective mulches have shown potential in increasing light distribution into the tree canopy and thereby increasing red color development (Leão de Sousa and Sánchez, 2020; Privé et al., 2008). Warm temperatures, on the other hand, are detrimental to the temperature-dependent anthocyanin biosynthetic pathway, where increased temperatures hinder sufficient red color development (Honda and Moriya, 2018; Gouws and Steyn, 2014), a trend which is becoming increasingly problematic as global temperatures rise.

Fruit ripening and red color development in almost all apples occur concurrently. In climacteric fruit such as apples, the ripening process is mediated by a rise in endogenous ethylene synthesis (Saure, 1990). Plant growth regulators (PGRs) such as methyl jasmonate (Shafiq et al., 2011), ethephon (Larrigaudiere et al., 1996; Li et al., 2002) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Van de Poel and Van Der Straeten, 2014) have been used to enhance ethylene production in fruit and subsequently stimulate red color development, although fruit ripening is also advanced. This may lead to decreased storability of fruit and the development of disorders such as diffuse internal browning (Butler, 2015). In order to counteract this effect, preharvest aminoethoxyvinylglycine (AVG), which competitively inhibits the activity of ACC synthase (ACS) and thus ethylene synthesis (Whale et al., 2008), or pre- or postharvest 1-methylcylopropene (1-MCP), which binds to ethylene receptor sites and thereby inhibiting its action, can be applied (Falagan and Terry, 2020; Tomala et al., 2020).

In the literature study, a broad overview of color development in apple is provided, including factors affecting color development and methods to improve red color development in apples. In the research chapters, two options to improve color development are investigated, viz., the use of plant growth regulators and reflective mulches.

In Paper 1 we report on the effect of ACC (200 $\mu L \cdot L^{-1}$) on red color development and fruit ripening of ‘Cripps’ Pink’ apples, and whether these are influenced by the application of AVG (125 $mg \cdot L^{-1}$) preharvest or 1-MCP postharvest. In Paper 2 we report the dose rate effect of preharvest foliar application of ACC (100 $\mu L \cdot L^{-1}$ – 400 $\mu L \cdot L^{-1}$) on ‘Fuji Kiku’ (Brak) and ‘Cripps’ Pink’ apples to increase red color development, in combination with a postharvest 1-MCP treatment to halt the ripening process. We also evaluate internal ethylene concentration of ‘Cripps’ Pink’ apples after ACC application until the first harvest. In Paper 3 we report on the use of two commercially available reflective mulches to increase red color development in ‘Fuji Brak’ (Kiku) apples in the Elgin region and ‘Rosy Glow’ apples in the Ceres region. We also report on the effect of the respective reflective mulches when used in combination with either draped or permanent shade net. Photosynthetically active radiation (PAR) was measured to determine to what extent the two mulches installed in the netted and open ‘Rosy Glow’ orchards affected PAR.

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LITERATURE REVIEW: An Overview of Red Color Development in Apple Fruit

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1. Introduction

The success of red and bi-color apple cultivars, such as 'Cripps' Pink', 'Fuji', 'Braeburn' and 'Cripps' Red', relies heavily on adequate red peel color development. Improved red color is often synonymous with higher income for producers of these apple cultivars, especially when they export their fruit. The favorable exchange rates in 2018 have meant an increase in Rand per ton from R 9 220 in 2017 to R 11 419 in 2018 (Hortgro, 2018), and although this fluctuate annually it remains a driving force encouraging producers to increase exports. The amount of apples exported from South Africa increased by 3.2% from 2017 to 2018, from 43.9% to 47.1% (Hortgro, 2018).

South Africa exported most of its apples to Africa (31%), followed by the Far East and Asia (32%) and thirdly to the United Kingdom (17%), Middle East (7%) and Europe (6%) in 2019 (Hortgro, 2019). Market demand varies across the world in terms of fruit characteristics and qualities. Generally, consumers seem to favor fruit which are redder (Csihon and Gonda, 2016; Hamadziripi et al., 2014) and in recent years blushed cultivars have become more popular.

An example of a successful blushed apple cultivar cultivated in South Africa is 'Cripps' Pink', which is sold under the trademark Pink Lady™, provided that it complies with certain requirements, including an average surface blush coverage of 40% or more (Dias and Tschantre, 2008), depending on the market. Originating from Western Australia, the global success of this cultivar is reflected in the increasing number of hectares planted in South Africa in recent years. It has become the fourth most planted cultivar in South Africa (Hortgro, 2018). However, the cultivar is known for inconsistent red color development, especially in the original strain of the cultivar resulting in low pack-outs. Red color is one of the most important factors when it comes to market acceptance of red and bi-color apples (Meng et al., 2015; Ritenour and Khemira, 1977; Saure, 1990; Whale et al., 2008). Major economic losses are experienced in regions where apples develop poor red color, as experienced in warmer production regions like South Africa, California and Australia. This is especially true for blush apple cultivars like 'Cripps' Pink', where a minimum blush color of 40%, market-depending, is required for apples to be considered first grade and fall under the premium priced trade name Pink Lady™ (Anon., 2020; Whale et al., 2008). Besides global cosmetic appeal, red or bi-colored apples are also associated with enhanced health benefits compared to their greener counterparts (Meng et al., 2015; Saure, 1990), as an increase in red color is associated with an increase in antioxidant compounds (Thomson et al., 2018).

It is thus of great interest to the apple industry to investigate red color development of apples to attain a better understanding of the physiological pathways involved. In this way, further investigations can be initiated to find methods of improving color development of especially high value red and bi-color apple cultivars.

2. Physiology of color development in apple fruit

Red color development in apples directly results from the synthesis and accumulation of anthocyanins and to a lesser degree the degradation of chlorophyll (Ritenour and Khemira, 1977). However, the most important ratio is that between chlorophyll and anthocyanins (Whale and Singh, 2007) and as ripening occurs and chlorophyll degradation takes place, the amount of anthocyanin in the peel of red cultivars has been shown to increase up to five times the original amount (Ritenour and Khemira, 1977). Thus, the authors state that optimization of factors that increase anthocyanin is of great importance, while the factors which lead to a decline in anthocyanin concentration must be minimized for optimum red coloration to occur. Timberlake and Bridle (1971), Saure (1990) as well as Thomson et al. (2017) agree that the main glycoside of anthocyanin found in the peel of ripe apples and is responsible for the red color, is cyanidin-3-galactoside (idaein). It was further established that two minor pigments, namely cyanindin-3-arabinoside and cyanidin-7-arabinoside, also play a role in the red color of apples.

Anthocyanin synthesis coincides with a peak in endogenous ethylene in apples (Whale and Singh, 2007). Thus, it can be deduced that factors increasing ethylene synthesis in fruit will allow for an increase in anthocyanin synthesis either directly or indirectly (Faust, 1973) and will lead to an increase in red color development of fruit.

Although there is significant genetic control over the formation of anthocyanins, various other factors also play a role in the pathways contributing to sufficient red color development in apple peel. Genetically, cultivars are predisposed to produce a certain type and color apple, and this is attributed to the fact that each step of the biochemical pathway of anthocyanin synthesis is genetically determined (Ritenour and Khemira, 1977). Anthocyanin biosynthesis regulation occurs mainly at the transcriptional level in apples (Meng et al., 2015). However, various environmental and management factors influence anthocyanin production (Gurnsey and Lawes, 1999). Among these environmental factors are light, which to some extent can be controlled by canopy management, temperature in the orchard as well as the stage of development of the fruit (Shafiq et al., 2011a). Management factors include cultural practices, such as thinning and pruning, fertilization, irrigation, and application of chemicals (Saure, 1990).

To fully comprehend color development in apples, the above-mentioned factors and their influence on anthocyanin biosynthesis must be holistically understood, and will thus be further discussed in the following sections in this review.

2.1. Anthocyanin formation and accumulation

As mentioned above, anthocyanins are the key pigments responsible for the development of red color in the peel of apples. Anthocyanins, synthesized in the epidermis and hypodermis of apple fruit peel (Barasu, 2017), are considered antioxidants that serve a photoprotective function to apple fruit (Gould, 1996). Both the amount and the composition of the anthocyanins contribute towards visible coloration of the apple peel (Shafiq et al., 2014). More specifically, the anthocyanin cyanidin-3-galactoside is responsible for red peel color in apples, and to a lesser degree, cyanidin-3-arabinosed and cyanidin-7-arabinoside (Marini, 2017; Saure, 1990; Shafiq et al., 2014; Takos et al., 2006). It can be added that these secondary metabolites are products of the flavonoid biosynthetic pathway (Thomson et al., 2018), of which the conversion of phenylalanine to p-coumaroyl-coA by the enzyme phenylalanine ammonia lyase (PAL) is the first committed step (Meng et al., 2015; Shafiq and Singh, 2018). PAL is thus the main enzyme playing a regulatory role in the synthesis of anthocyanins via coumaric and cinnamic acids from the amino acid phenylalanine (Barasu, 2017; Jiang and Joyce, 2003).

Both internal and external stimuli affect the anthocyanin biosynthetic pathway and thus red color development during fruit ripening. Although genetic composition is the main determinant of anthocyanin biosynthesis (Thomson et al., 2018), the influence of climate should not be underestimated (Barasu, 2017; Pearce and Streeter, 1931). Temperature and especially light affect anthocyanin formation (Meng et al., 2015). Increased exposure of fruit to light as well as a decrease in temperatures are suggested to increase PAL activity in apple fruit (Blankenship and Unrath, 1988; Ritenour and Khemira, 1977). The assimilates produced during photosynthesis are essential to anthocyanin biosynthesis (Barasu, 2017; Shafiq et al., 2014) and further supports the emphasis on light requirement for adequate red color development. The plant hormone ethylene also has a stimulating effect on PAL activity (Whale and Singh, 2007). According to Whale and Singh (2007), an increase in PAL activity directly leads to an increase in the amount of anthocyanin accumulated within the apple peel. Thus, increased light exposure, exposure to ethylene and low temperatures favor anthocyanin biosynthesis, although the extent to which each stimulus influences this biosynthesis is not fully understood.

According to Saure (1990), two peaks of anthocyanin formation exist in apples. The first peak, being economically insignificant, occurs early in fruit development during the period of intense cell division. The second peak coincides with a rise in respiration and endogenous ethylene during fruit ripening. This is especially significant for red and bi-color apple cultivars (Whale and Singh, 2007). In addition to this, it is established that with an increase in apple maturity, there is an increase in capacity of the fruit to accumulate anthocyanin, since the rate of anthocyanin synthesis is greater than anthocyanin degradation after maturation of apple peel (Saure, 1990). Furthermore, Steyn et al. (2009) reported that throughout fruit development, red color in 'Cripps' Pink' apples increased rapidly with a decrease in temperature, but color subsequently also decreased rapidly with an increase in temperature. This indicates the importance of temperature on color development and the fluctuating nature of red color in apple peel.

It is apparent that various factors affect anthocyanin biosynthesis and accumulation. Among these are genetics, fruit maturity, light, and temperature. It is important to implement proper management strategies to manage the above-mentioned factors to optimize anthocyanin formation and accumulation, and in doing so, also optimize red color development in the peel of apples.

2.2. Chlorophyll degradation

As fruit mature and ripen, there is a subsequent decrease in chlorophyll concentration in the peel of apples, which results in a decreased chlorophyll to anthocyanin ratio (Whale and Singh, 2007). Whale and Singh (2007) ascribe the phenomenon of chlorophyll degradation to photosynthetic units that disintegrate with fruit ripening. This means that chloroplasts are converted to chromoplasts and the thylakoid membrane is degraded to form plastoglobuli. As chlorophyll decreases, anthocyanins and thus red color becomes more visible (redder, lighter and brighter) as ripening progresses.

A decrease in the chlorophyll concentration in apple peel is also strongly correlated to an increase in endogenous ethylene synthesis (Whale and Singh, 2007). Iqbal et al. (2017) stated that parameters such as color changes involving an increase in anthocyanins and carotenoids and a decrease in chlorophyll concentration in climacteric fruit are regulated by the plant hormone ethylene. Thus, exposure to ethylene will aid in chlorophyll degradation and red color development of apples.

3. Factors affecting color development

There are various factors that affect color development in apples. The three main groups of factors that will be discussed below are genetic and developmental factors, environmental factors, and finally factors pertaining to canopy management. Although individually each of these factors are essential, it is important to note that the combination of all these factors is required for maximum color development to occur. It is therefore necessary to view the role that these factors play in color development holistically, although they will be discussed individually.

3.1. Genetic and developmental factors

It is widely reported that color development varies among apple cultivars and that genetic composition is an important determinant of the fruit's predisposition to develop red color (Thomson et al., 2018). The predetermined color of apple fruit relies heavily on its genetic composition, which includes the alleles of genes necessary for anthocyanin synthesis as well as its precursors (Honda and Moriya, 2018). In apples, *MdMYB1* is the dominant gene responsible for red pigmentation of the fruit peel and this gene is light responsive (Dar et al., 2019; Honda and Moriya, 2018). The degree to which color development is induced by color promoting factors such as exposure to light and low temperatures varies greatly among cultivars (Ritenour and Khemira, 1977). Gurnsey and Lawes (1999) thus emphasizes the importance of cultivar selection with the final product and amount of color development desired in mind.

Together with the cultivar, the stage of development of the fruit as well as the tree is another important factor for the degree of sensitivity fruit display towards color development (Iqbal et al., 2017; Ritenour and Khemira, 1977). Considering that apples are climacteric fruit and that color development is associated with a substantial increase in the production of ethylene with ripening, fruit must be horticulturally mature enough, reaching climacteric at an internal ethylene concentration (IEC) equivalent to more than 1 $\mu l \cdot L^{-1}$ (Doerflinger et al., 2019), to be ethylene sensitive and for anthocyanin synthesis to occur (Iqbal et al., 2017; Ma et al., 2014; Shafiq et al., 2011a). Thus, as apples mature, increased internal ethylene concentrations possibly increase the activity of PAL as well as the rate at which anthocyanin is synthesized (Li et al., 2002; Saure, 1990), although the rate at which ethylene is produced differs to some extent among cultivars (Musacchi and Serra, 2018). Furthermore, maturity of fruit often differs within the same tree. Musacchi and Serra (2018) found more mature fruit in the lower parts of the tree canopy and less mature fruit found towards the top of the tree canopy. However, increased light exposure of the top fruit often causes fruit found at this position to display substantially more red color development than fruit lower in the canopy that may be shaded by both other fruit and foliage.

It is evident that although the genetic composition and developmental stage of the apple fruit are among the most important factors regulating anthocyanin development and accumulation, a combination of various favorable factors are required for maximal red color development.

3.2. Environmental factors

Following the genetic composition, environmental factors has the second-largest influence on red color development in fruit. This is especially true for fruit exposure to light. Other factors that will be discussed in this section include the influence of temperature as well as that of soil on the development of color in apple peel. Once again, it is of value to consider the role that environmental factors play on color development holistically, as it interacts and is closely linked to genetic factors as well as management factors.

3.2.1. Light irradiation

Light is one of the most important factors that influence red color development of apples and can be controlled to a certain degree by pruning and other orchard management practices like leaf removal around the fruit (Palmer et al., 1992). Although the optimal amount of light required for the most red color development relatively depends on the cultivar and the stage of development of the fruit, both the quantity as well as the quality of light is of significance. Fruit exposure to light stimulates PAL activity in the apple peel, up-regulating anthocyanin biosynthesis and red color development (Blankenship and Unrath, 1988).

According to Ritenour and Khemira (1997), light interception of more than 70% is best for red color development, with less than 40% delivering less favorable results, still bearing in mind that the light requirement of each cultivar may differ. Later, Gurnsey and Lawes (1999) agreed that 70% light will cause superior red coloration, with 50% light exposure producing adequate results; warning that excessive light exposure poses the risk of sunburn of fruit. Various authors agree that UV and UV-B light are the most effective wavelengths for fruit color development, although visible light is also effective (Musacchi and Serra, 2018; Ritenour and Khemira, 1977). Orchard management practices that can be utilized to maximize light exposure to fruit include leaf removal and branch pruning and installing reflective mulch. Hamadziripi et al. (2014) reported that in a study on ‘Granny Smith’ apples, fruit from the inner canopy were exposed to a low average of 2% of the seasonal photosynthetic photon flux from sunlight, whereas fruit from the outer parts of the tree canopy received 54% of average photosynthetic photon flux over the season. This is supported by Kays (1999), specifically on red apple cultivars. There has been a substantial increase in the use of hail nets over high value crops such as bi-color apples in recent years (Weber et al., 2019). This lowers irradiation levels reaching fruit and thus decreasing anthocyanin biosynthesis. Low levels of irradiance are also associated with smaller fruit (Kays, 1999; Musacchi and Serra, 2018). Not only does light have a direct effect on stimulating anthocyanin biosynthesis, but it also favors photosynthesis, resulting in increased assimilates available for anthocyanin synthesis (Barasu, 2017).

It is evident that light plays a vital role in anthocyanin biosynthesis, both directly and indirectly. The degree to which light affects color development, however, remains heavily cultivar dependent (Tijskens et al., 2011).

3.2.2. Temperature

It is widely reported that temperature is among the most important factors affecting red color development in apples, and throughout literature there is a clear understanding that lower temperatures enhance anthocyanin biosynthesis and promote red color development (Gouws and Steyn, 2014; Ritenour and Khemira, 1977; Saure, 1990; Warrington et al., 1999; Whale et al., 2008). This explains, to some degree why poor and erratic color development is so prevalent in red and bi-color apples in regions of the world with warmer climates, such as the Western Cape Province in South Africa, Western Australia and some Mediterranean regions (Shafiq et al., 2011b). Locally, producers can testify to this when comparing apples from a slightly warmer Elgin region to those from a cooler Koue Bokkeveld region where lower night air temperatures result in greater induction in anthocyanin synthesis and red color development (Gouws and Steyn, 2014).

According to Gurnsey and Lawes (1999), temperature affects red color development most between two to three weeks prior to harvest and day temperatures should fall within the range of 20-25°C. However, this range varies between cultivars. This statement is supported by Saure (1990) and Ritenour and Khemira (1997) who add that the stage of development of fruit also plays a role in the effect temperature has on red color development. Gouws and Steyn (2014) on the other hand, extend this period to a full month before harvest. These authors ascribe this to the fact that this is the time when a peak in anthocyanin synthesis is observed in South African cultivars, a statement which is verified by Saure (1990). In addition to this, Gouws and Steyn (2014) specify that a temperature of 16 °C to 25 °C is the range in which maximal red color development occurs in 'Cripps Pink' apples. Red color also has the tendency to fluctuate with fluctuating temperature, as reported by Steyn et al. (2009). A decrease in temperature, such as with a cold front, leads to a sudden increase in red color development, but an increase in temperature between cold fronts subsequently leads to a rapid fading of red color in the apple peel of bi-color apples.

Cooler air temperatures at night are known to increase red color development (Blankenship, 1987). Gurnsey and Lawes (1999) specify that night temperatures below 18 °C are favorable. This may be due to a decrease in respiration with lower temperatures, leading to fewer sugars being lost by the apple peel, resulting in an increased amount of sugar substrate available for anthocyanin synthesis (Ritenour and Khemira, 1977). Another possibility is that decreased night temperatures result in a significant increase in the transcription of genes relevant to the biosynthetic pathway of anthocyanin (Lin-Wang et al., 2011). However, many authors agree that even a single day of high temperatures can negate the beneficial effect of the colder night before (Ritenour and Khemira, 1977; Saure, 1990). According to Gurnsey and Lawes (1999), temperatures reaching 30 °C or more during the day will negate the positive effect of the cool temperature of the prior night. Temperatures fluctuating between cool night air temperatures and moderate day air temperatures are therefore most beneficial to optimum red color development (Lin-Wang et al., 2011), a finding also supported by Reay (1999), who found that exposure of 'Granny Smith' apples to a temperature combination of 4 °C in the dark for and 20 °C in UVB-Visible light for 16 and 75 hours respectively, increased anthocyanin concentration present in the apple peel. Barasu (2017) also found that uninterrupted cooling periods preceding harvest are most beneficial for red color development.

Temperature therefore plays a major role in anthocyanin synthesis and red color development. The specific region where apples are produced is thus important, as this determines the climate and ultimately the general temperature trends of the area (Gurnsey and Lawes, 1999), and can be used in deciding on which cultivar will be best suited to the relevant environment.

3.2.3. Soil and foliar nutrition

Soil nutrition is not only important for overall tree health (Musacchi and Serra, 2018), but can also play a role in color development of fruit. This includes not only soil nutrients such as nitrogen (N), potassium (K) and others, but also soil pH.

It has been found that surplus N fertilization can lead to a decrease in anthocyanin synthesis and thus red color development (Ritenour and Khemira, 1977). Although there may be an increase in overall yield of well-colored fruit, Saure (1990) agrees that excessive N application correlates to a lower percentage of fruit which are well-colored at harvest. This can be a direct effect of N or be attributed to an increase in N leading to an abundance in shoot production. As more shoots develop, fruit are more shaded, thus inhibiting anthocyanin formation (Gurnsey and Lawes, 1999). An increase in vegetative growth also implies that less light penetration can occur into the tree canopy, thereby also preventing anthocyanin biosynthesis (Ritenour and Khemira, 1977). Higher N levels late in the growing season are the most detrimental to red color development (Saure, 1990), as this is the most active period of anthocyanin synthesis (Gouws and Steyn, 2014). However, maintaining very low rates of N can have a detrimental effect on fruit and trees, as there is the possibility of alternate bearing of trees and a decrease in fruit size (Ritenour and Khemira, 1977). Thus, a middle ground of N fertilization should be found that optimizes tree health but does not negatively affect red color development of fruit.

K and N are antagonistic elements. It is therefore not surprising that higher levels of K increase anthocyanin production (Saure, 1990). The author states that a high level of K compensates for the negative effect of high levels of N rather than having a direct effect on color development per se. This statement is supported by Ritenour and Khemira (1997), who also add that high levels of K possibly stimulate red color development by promoting normal and healthy fruit development. Ritenour and Khemira (1997) also found that for soil pH ranging from 4.1 to 6.9, an increase in red color and fruit size was observed in ‘Red Delicious’ at the higher pH.

Nutritional status of the plant can positively influence color development of fruit (Larrigaudiere et al., 1996). Important minerals to consider are nitrogen (N) and calcium (Ca). Excessive N fertilization may increase vegetative growth causing less light penetration into the canopy and less color development of fruit (Gurnsey and Lawes, 1999). However, Ritenour and Khemira (1997) warns that applying rates of N that are too low can cause fruit size to suffer and trees to experience alternate bearing. The role of Ca is as secondary messenger in the plant (Li et al., 2002) which may lead to increased anthocyanin expression.

Overall, adequate soil nutrition is invaluable not only for red color development, but for general tree health and should be closely monitored and adjusted if needed.

3.3. Management factors

For the purposes of this discussion, the term canopy management is used broadly to encompass various elements, including orchard establishment, pruning, thinning, irrigation and fertilization. Extensive research has been done on optimizing management practices. Since red color development is heavily dependent on light exposure, any managerial practice optimizing this will increase red color development (Stern et al., 2010; Thomson et al., 2018).

Factors to consider when establishing an orchard therefore not only includes consideration of the cultivar to be planted, but also the rootstock used. A dwarfing rootstock, for example, will ultimately result in a smaller tree and less foliage to shaded fruit (Ritenour and Khemira, 1977), therefore leading to increased red color development. The opposite is true for a rootstock promoting more vigorous vegetative growth. The row direction, planting density and training system of orchard trees are also of importance in ensuring optimum exposure of fruit to sunlight (Javaid et al., 2017).

Pruning in summer can also increase light penetration and distribution into the canopy (Musacchi and Serra, 2018). It is important to manage the style and severity of pruning to ensure that sufficient light penetrates the canopy, but not so much that fruit experience sunburn. Generally, the inner parts as well as the lower parts of the canopy are least exposed to sunlight and typically experience less red color development (Weber et al., 2019), but to a lessor extent on dwarfing rootstocks. Ritenour and Khemira (1997) regards the leaf to fruit ratio as very important, not only for preventing sunburn, but also for ensuring sufficient photosynthate availability per fruit. An increased leaf to fruit ratio in turn ensures more sugars are available to fruit for anthocyanin synthesis. Crop load also plays a role in sugar availability and thus fruit thinning also helps maintain a balanced leaf to fruit ratio (Gurnsey and Lawes, 1999). In this way, not only blush color is maximized, but fruit size is also improved.

It is apparent that canopy management is an important point of control where producers can influence color development depending on their chosen management practices. It is thus noteworthy to investigate and optimize cultural practices and canopy management to make accurate and informed recommendations to both future researchers as well as producers.

4. The role of ethylene in red color development

Ethylene is a gaseous phytohormone which regulates various developmental and biochemical pathways in fruit. This includes pathways which affect fruit ripening, nutritional quality, color, texture and aroma (Ireland et al., 2014; Li et al., 2002). Ethylene is also involved in stimulating fruit abscission (Stern et al., 2010). Although ethylene synthesis of fruit as well as the sensitivity of fruit to ethylene vary among cultivars and even individual trees (Musacchi and Serra, 2018), Bhadoria et al. (2018) has shown that the ripening process of climacteric fruit is initiated by ethylene at an internal ethylene concentration ranging from 0.1 $\mu l \cdot L^{-1}$ to 1.0 $\mu l \cdot L^{-1}$. Climacteric fruit are fruit which display a characteristic coinciding peak in endogenous ethylene with the peak in respiration as fruit ripening occurs (Blankenship and Unrath, 1988; Whale and Singh, 2007). Examples of climacteric fruit are apple, tomato, peach, avocado and banana (Ireland et al., 2014; Müller and Stummann, 2003). Ethylene synthesis is autocatalytic (Handa et al., 2012; Musacchi and Serra, 2018), and is also stimulated by exposure to exogenous ethylene sources (Müller and Stummann, 2003).

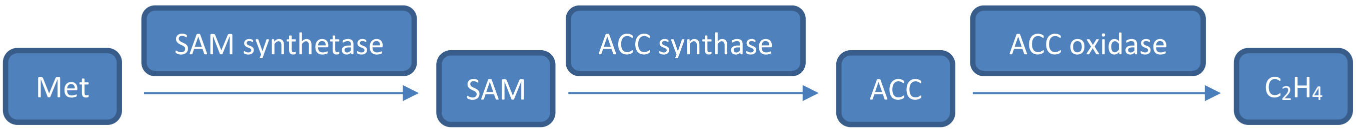
One of the most effective methods of either promoting or delaying the ripening process of climacteric fruit is to manipulate ethylene production and exposure (Zhu et al., 2015). Promoting ripening could lead to increased anthocyanin biosynthesis (Shafiq et al., 2014) and increased red color development and visual appeal of fruit (Faust, 1973). This can be achieved by exposing fruit to compounds which stimulate ethylene biosynthesis or compounds which are ethylene releasing. A compound which is widely used in the apple industry is the ethylene releasing product, ethephon (Shafiq et al., 2014). Delaying ripening by inhibiting ethylene biosynthesis, on the other hand, can prolong fruit shelf-life (Iqbal et al., 2017). Chemical inhibitors can successfully and efficiently suppress ethylene synthesis (Ireland et al., 2014). An example of an ethylene inhibiting compound is the cyclic alkene 1-methylcyclopropane (1-MCP), which binds to ethylene receptor sites (Zhu et al., 2015).

It is important to consider timing of application of such compounds, since ethylene sensitivity of fruit is strictly regulated by developmental factors (Ma et al., 2014). Immature fruit do not produce vast amounts of endogenous ethylene and are also insensitive to exogenously applied ethylene (Iqbal et al., 2017), making any ethylene treatments ineffective. As climacteric fruit ripen, however, ethylene synthesis increases by as much as 1000 fold (Musacchi and Serra, 2018). Although non-climacteric fruit, such as grape and citrus, do not display the same spike in endogenous ethylene production as the fruit ripens, ripening will still progress in some non-climacteric fruit if the fruit is exposed to an exogenous ethylene source (Osorio et al., 2013). Ethylene is essential for satisfactory fruit ripening and postharvest quality and is of significant commercial importance (Adams and Yang, 1981).

4.1. Ethylene biosynthetic pathway

The ethylene biosynthesis pathway is relatively simple and linear and has been thoroughly researched and has established a comprehensive understanding of how ethylene production occurs in plants (Adams and Yang, 1981).

There are three enzymatic reactions involved in the pathway, as illustrated in the schematic below:



The first reaction consists of the activation of methionine (Met) to S-adenosylmethionine (SAM). This occurs via the enzyme SAM synthetase. Step two follows where SAM is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by means of the enzyme ACC synthase (ACS). Finally, oxygenation of ACC occurs via ACC oxidase (ACO) to produce ethylene as the end product (Adams and Yang, 1981; Ma et al., 2014).

It is widely reported that the second step in the pathway is the rate-limiting step in ethylene biosynthesis (Adams and Yang, 1981; Handa et al., 2012; Ma et al., 2014; Van de Poel and Van Der Straeten, 2014). In addition, fruit developmental, environmental signals as well as hormonal signals all play a major role in regulating the level of ethylene in plant tissues (Ireland et al., 2014). This regulation occurs via controlling the enzymes involved in the pathway, ACS and ACO, as well as by affecting fruit specific polygalacturonase (Iqbal et al., 2017). This regulation occurs either at transcriptional or protein levels (Ma et al., 2014).

There is a noticeable peak in ethylene biosynthesis during the ripening stage in climacteric fruit such as apple, compared to a basal amount of ethylene produced during the pre-climacteric period. As fruit enter the post-climacteric ripening stage, ethylene production decreases rapidly as a result of a decrease in ACO activity, while ACS activity remains constant (Ma et al., 2014). Ma et al. (2014) suggest that the reason for this is to ensure adequate seed maturation by preventing premature fruit senescence. This emphasizes the important role that the developmental stage of the fruit has on the biosynthesis of ethylene.

Ethylene is evidently a very influential hormone in terms of agriculture and affects many fruit and crop quality parameters, such as color development, textural changes, accumulation of sugars and acids, as well as aroma development (Jiang and Joyce, 2003). It is thus worthwhile and very useful to have a clear understanding of the pathways associated with the biosynthesis of this hormone, as well as the practical implications and uses that it may have for producers.

4.2. 1-Aminocyclopropane-1-carboxylic acid (ACC) as precursor to ethylene

As seen in the biosynthetic pathway of ethylene referred to above, ACC is the intermediate amino acid between methionine and ethylene. This cyclic amino acid, although quite simple in structure, plays an important role in the formation of ethylene (Van de Poel and Van Der Straeten, 2014).

ACC is synthesized from s-adenosyl methionine (SAM) by the enzyme ACC synthase (ACS) (Ma et al., 2014). During this conversion, 5’-methylthioadenosine (MTA) is also produced (Handa et al., 2012), and is reincorporated via the Yang cycle as part of methionine

(Müller & Stummann, 2003) during oxidation of ACC into ethylene by the enzyme ACC oxidase (ACO) (Adams & Yang, 1981). The well-established rate-limiting step of this biosynthetic pathway is the synthesis of ACC from SAM, catalyzed by ACS (Adams and Yang, 1981; Çetinbaş et al., 2012; Handa et al., 2012). This allows for a point of regulation within the pathway (Ma et al., 2014). One such example would be to up-regulate ethylene synthesis in fruit by applying exogenous ACC (Adams and Yang, 1981; Van de Poel and Van Der Straeten, 2014). ACC occurs naturally in plant tissues (Schupp et al., 2012) and is both easily translocated within these tissues and over longer distances within the plant via the xylem or phloem (Van de Poel and Van Der Straeten, 2014). ACC is also oxidized to ethylene readily throughout a large variety of plant tissues (Schupp et al., 2012). However, timing of ACC application is a very important point to consider as it affects the desired result. Applying ACC early in the season, when fruit are between 10 and 20 mm in diameter, has proven to be an effective chemical thinning agent in apple (Schupp et al., 2012), whereas application later in the season has potential to affect color development through up-regulation of ethylene synthesis. A recent paper also implicated that during bloom ACC is involved in pollen tube growth as it promotes secretion of pollen tube chemoattractant (Mou et al., 2020).

5. Methods of improving color development

There are several strategies for improving apple color development, albeit some traditional and some still in development. The discussion to follow will focus mainly on increasing red color development in apple fruit by chemical application but will briefly touch on some other effective strategies as well, such as pruning, thinning and reflective mulch.

5.1. Chemical application

Various chemical compounds have been shown to be effective enhancers of red color development in apples. One of the most well-researched compounds is ethephon, an ethylene-releasing product that promotes ripening of fruit (Gurnsey and Lawes, 1999; Li et al., 2002; Whale and Singh, 2007; Zhu et al., 2015). Theoretically, using this compound to enhance ethylene production in fruit should be plausible, since ethylene biosynthesis is autocatalytic in the case of climacteric fruit (Müller and Stummann, 2003). Furthermore, stimulating ethylene biosynthesis initiates fruit ripening (Faust, 1973), a process directly linked to red color development in apples. Laurrigaudiere et al. (1996) applied ethephon to ‘Starking Delicious’ apples approximately two to three weeks before harvest and found that not only did it improve the color of the treated apples, but there was also a considerable increase in PAL as well as ACO enzyme activity in the fruit peel. Both these enzymes play significant roles in red color development. An increase in ACO leads to increased endogenous ethylene, which favors color improvement of fruit indirectly via fruit ripening, whereas an increase in PAL activity may stimulate anthocyanin biosynthesis directly. However, the authors also noted that the ethephon treatment stimulated ripening, which was a less desirable outcome. Gurnsey and Lawes (1999) reported that the same side-effect occurred when ethephon was applied one to three weeks before harvest at rates between 100 and 600 $g \cdot L^{-1}$, with the accelerated ripening causing fruit to lose firmness and therefore deteriorate eating quality. Li et al. (2002) found that applying ethephon to ‘Fuji’ apples four weeks before harvest increases red peel color, the concentration of flavonoid compounds, ACO and PAL activity, as well as internal ethylene concentrations (IEC). However, they added that the mechanism by which the up-regulation of these enzymes occur by low temperatures, and therefore the subsequent coloration of the apple fruit, is not yet fully known. According to Bhadoria et al. (2018), ethephon also has potential hepatotoxic qualities resulting in symptoms in humans like diarrhea, salivation, and stomach cramps. The toxicity of ethephon should be further investigated to ensure its safety for use on fresh produce, not only for human ingestion, but also for pollinating insects (Schupp et al., 2012).

To counter the detrimental ripening effect of ethephon, Whale et al. (2008) applied a combination of ethephon and aminoethoxyvinylglycine (AVG) to ‘Cripps Pink’ apples. AVG causes a delay in ripening by preventing ethylene biosynthesis by competitively inhibiting ACS activity (Çetinbaş et al., 2012). Çetinbaş et al. (2012) noted that while ethephon caused an increase in the percentage of red blush on fruit while increasing respiration rate, and AVG caused the opposite result, a combination of ethephon and AVG treatments successfully increased red blush coloration compared to control treatments without affecting respiration rate of apple fruit. Furthermore, fruit firmness as well as internal ethylene (IE) remained unaffected in the combination treatments, whereas IE increased in apple fruit treated with ethephon. The compound 1-methylcyclopropane (1-MCP) has been used to achieve similar results to AVG (Handa et al., 2012; Hughes and Dickerson, 1989; Zhu et al., 2015). The mode of action by which 1-MCP inhibits ethylene biosynthesis is by blocking ethylene receptor sites (Handa et al., 2012). It can be applied pre-harvest in order to decrease IE of fruit, as proven by Tomala et al. (2020) on ‘Szampion’ apples in Poland, or postharvest as shown by Falagan (2020) on various apple cultivars to maintain fruit quality with long term storage.

Other substances which have shown promising results in terms of fruit coloration is the synthetic auxin 2,4-DP on 'Cripps' Pink' apples (Stern et al., 2010), mineral phosphorous-calcium mixture, seniphos, on 'Starking Delicious' apples (Larrigaudiere et al., 1996), methyl jasmonate on 'Cripps' Pink' apples (Shafiq et al., 2011b) and ACC (Van de Poel and Van Der Straeten, 2014), all of which are reported to enhance ethylene production. Externally applied phenylpropanoids (Shafiq and Singh, 2018) and abscisic acid (ABA) (Jiang and Joyce, 2003) have also been shown to increase red color development through up-regulation of the anthocyanin biosynthetic pathway in 'Cripps' Pink' apples and strawberries, respectively.

5.2. Reflective mulch

Reflective mulch, although a relatively expensive option (Gurnsey and Lawes, 1999), can significantly increase light exposure and color development of fruit, especially under lowered light conditions such as under shade nets. Weber et al. (2019) managed to reflect between 1.6 and 3.9 times more photosynthetically active radiation (PAR) and ultra-violet (UV) light at one meter above the ground using Lumilys® reflective ground cover compared to the grassed alleyway used as control in a 'Braeburn Mariri Red' orchard. The authors report a significant improvement in fruit peel color, especially regarding areas in the canopy where poorly colored fruit is typically found, such as the inner and lower parts of the tree canopy. Privé et al (2008) similarly reported a five to nine times level of reflected PAR into the lower canopy using Extenday® reflective fabric in a 'Gala' apple orchard in Canada. PAR was measured over the growing season, and a significant increase in PAR was also noted in the middle and upper tree canopy. Reflective mulches therefore increase diffuse light availability in the whole tree canopy. This suggests that reflective mulches not only have the potential to increase red color development through upregulation of anthocyanin synthesis but may also have a positive effect on net photosynthesis of the tree and assimilate availability (Leão de Sousa and Sánchez, 2020).

Gurnsey and Lawes (1999) emphasize the relatively high cost of reflective mulches, but also state that mulches can be installed as late as one month before harvest. This makes it possible for the same mulch to be used for multiple cultivars and since it should last over multiple seasons, the cost is justified. An increase in fruit color may also lead to greater profits, further justifying the initial high input costs of mulches. Overbeck et al. (2013) reported an increase in the percentage of fruit with more than 75% color coverage from 81.7% for the uncovered control to 95.6% for Extenday® in a 'Gala Mondial' orchard in Germany.

The effect of reflective mulch on fruit maturity varies in the literature, where some authors report advancement of maturity with reflective mulch installation (Overbeck et al., 2013) and others report retarded ripening of fruit with mulch installation (Schuhknecht et al., 2018). The influence of reflective mulch on fruit maturity should thus be further investigated.

5.3. Other practices

Alternative methods to improve coloration of apples include factors involved in orchard establishment, and canopy management practices such as pruning and thinning, defoliation, evaporative cooling and delaying harvest. Site and cultivar selection are two important factors to take into consideration when establishing an orchard. The orchard site will determine the microclimate and soil conditions under which fruit will be cultivated (Gurnsey and Lawes, 1999; Kays, 1999). Since the soil is the major source of plant nutrients, choosing a rootstock that is well adapted to ensure optimum mineral and water uptake in that environment is crucial (Valverdi et al., 2019). Since various macronutrients needed for plant and fruit development are taken up by mass flow into plant roots, irrigation should be optimized (Valverdi et al., 2019). Included in these macronutrients are nitrogen (N), phosphorus (P) and potassium (K). Optimizing the soil environment will aid in ensuring optimal overall tree and fruit health, which is ultimately beneficial to fruit color development.

Pruning of branches and defoliation can be effective strategies to optimize light interception into the tree canopy and onto fruit surfaces, resulting in increased fruit coloration (Pearce and Streeter, 1931). However, caution should be taken with pruning severity as a low leaf to fruit ratio can lead to insufficient photosynthate availability for anthocyanin synthesis (Gurnsey and Lawes, 1999; Ritenour and Khemira, 1977). Thinning apple fruit also has a positive effect on color development. Gurnsey and Lawes (1999) suggests that clusters of even two to three fruit can be detrimental to color development. Shü et al. (2001) found that trees with heavy crop loads tend to produce poorly colored apples. This is attributed to both overshadowing by neighboring fruit as well as limited sugar availability for anthocyanin production and optimal fruit development. Leaf removal by hand or machines are also an option. When removing leaves manually, leaves around fruit are removed to improve the light exposure of the fruit surface. In addition, evaporative cooling of fruit can be considered when water of sufficient quality is available. Iglesias et al. (2002) found increased red color, and higher anthocyanin content with microsprinkler irrigation on ‘Topred Delicious’ apples, especially when applied at sunset and at sunrise.

Another proposed method for increasing color development of apple fruit is delaying commercial harvest (Whale and Singh, 2007). Shafiq et al. (2011a) successfully increased red blush color of ‘Cripps Pink’ apples by delaying harvest by two to six weeks after commercial maturity. In the study, both the anthocyanin concentration of the fruit and the percentage of export grade pack-out increased compared to the control. Unfortunately, together with the increased ethylene concentration observed, fruit firmness also declined as fruit maturity was advanced. Delaying fruit harvest thus poses a risk to fruit quality and storability.

Although isolated remedies seem to be able to have potential to increase color development of apples, it is always important to bear in mind that activities in the orchard are interdependent and one will tend to affect the other (Barasu, 2017).

6. Conclusion

It is economically beneficial for South African apple producers to have a high Class one pack-out accepted for export. In terms of red and bi-color cultivars, red color coverage of the fruit surface plays a major role in fruit grading and market acceptance. However, South African climatic conditions often pose challenges to achieve good color development. High temperatures during the summer months as well as insufficient light exposure to fruit are major contributors to inconsistent color development. Pruning and thinning of tree canopies has shown to be effective in improving light conditions leading to increased red color development. However, sunburn poses a serious risk in situations where very severe pruning is implemented and these practices also increase labor cost. In addition, reflective mulch can be used to increase irradiance into especially the lower parts of the tree canopy where poor fruit coloration is often experienced. Alternatively, chemical applications of especially ethylene enhancing compounds, such as ethephon, has proven to be very promising. Although it not only advances fruit ripening, it also poses some health hazards and phytotoxicity. There is thus an opportunity for further research into alternative methods to improve color development of high value bi-color apple cultivars to provide solutions to this problem to the apple industry.

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PAPER 1: The Effect of Preharvest Aminoethoxyvinylglycine and 1-Aminocyclopropane-1-carboxylic acid and Postharvest 1-Methylcylopropene on Red Color Development, and Quality of 'Cripps' Pink' Apples

Additional index words. Fruit color, *Malus domestica* Borkh., plant growth regulator, ethylene

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Abstract.

'Cripps' Pink' bi-color apples (*Malus domestica* Borkh.) grown in the warm Mediterranean-type climate of the Western Cape Province, South Africa suffer from poor and erratic red color development due to unfavorable temperature conditions. These apples are harvested based on percentage red blush coverage over multiple harvests. Often fruit from the lower and inner canopy, which are insufficiently exposed to sunlight for optimum color development, are harvested later to allow for these apples to color. This, however, results in fruit that are post-optimum maturity at harvest, leading to decreased storability and the risk of disorders such as diffuse internal browning. The purpose of this study was to determine the effect of 1-aminocyclopropane-1-carboxylic acid (ACC) (200 $\mu L \cdot L^{-1}$), aminoethoxyvinylglycine (AVG; ReTain®) (125 $mg \cdot L^{-1}$) and a combination of the two at two weeks before harvest (wbh) and one wbh on red peel color development and maturity of 'Cripps' Pink' apples. One ACC treatment was followed by a postharvest treatment of 1-methylcyclopropene (1-MCP). ACC application increased red blush coverage of fruit and fruit marketable as Pink Lady™ compared to the control. AVG had the opposite effect on red color development, with percentage blush coverage significantly lower than that of the untreated control. The combination of ACC and AVG resulted in red color coverage similar to that of the untreated control. Although fruit from all treatments were harvested at post optimum maturity, indicated by starch breakdown at harvest greater than 30%, percentage starch breakdown of AVG-treated fruit was consistently and significantly lower than ACC-treated fruit and the control. After storage at -0.5 °C for 12 weeks and seven days simulated shelf-life, the effect of 1-MCP on

maintaining fruit firmness and ground color was evident. This confirms that 1-MCP application postharvest delayed fruit ripening during cold storage.

In 2018, 'Cripps' Pink' apples (*Malus domestica* Borkh.) accounted for 12% (3 063 ha) of the total area planted to apples in South Africa (Hortgro, 2019), emphasizing the economic importance of the cultivar to the South African apple industry. These apples are sold under the tradename Pink Lady™, if they have a red-colored surface area of at least 60% for Asian and Gulf State territories and 40% for all other markets (Anon., 2020). High autumn temperatures in the Mediterranean climate cause insufficient red colour development. The original strain of 'Cripps' Pink' is notorious for poor and erratic red color development (Shafiq et al., 2011), limiting producer profits. Subsequent strains such as 'Rosy Glow' and 'Lady in Red' that have been selected for improved red color result in higher income per hactare. Substantial hectarages of the original strain still exist in the South African apple industry and these are harvested over multiple picking dates based on fruit red color. The fruit that are left on the tree longest are usually in the lower and inner canopy, which do not color as well as they are usually not well exposed to sunlight. The inner and lower canopy fruit are also more mature than their upper, outer canopy counterparts (Feng et al., 2014), and are often harvested post optimum maturity. This may lead to problems with long-term storability and increased risk of disorders such as diffuse internal browning (Butler, 2015).

Generally, fruit ripening and red color development in almost all apples occur concurrently. In climacteric fruit such as apples, the ripening process is mediated by a rise in endogenous ethylene synthesis and respiration (Saure, 1990). Plant growth regulators (PGRs) such as methyl jasmonate (Shafiq et al., 2011), ethephon (Larrigaudiere et al., 1996; Li et al., 2002) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Van de Poel and Van Der Straeten, 2014) have been used preharvest to enhance ethylene production in fruit and subsequently stimulate red color development in apples. With an increase in ethylene, there is a significant risk of fruit being overripe at harvest. To prevent this, aminoethoxyvinylglycine (AVG), which competitively inhibits the activity of ACC synthase (ACS), can inhibit ethylene biosynthesis (Whale et al., 2008). Similarly, 1-methylcylopropene (1-MCP), which binds to ethylene receptor sites and thereby inhibiting its action, can be applied either before harvest (Tomala et al., 2020) or as a postharvest treatment (Falagan and Terry, 2020). Postharvest 1-MCP application is a common practice in the apple industry (Watkins, 2009). Reducing ethylene synthesis allows fruit to be harvested at optimum maturity as well as for increased long-term storability of fruit without compromising fruit quality.

The aim of this study was to determine the effect of ACC on 'Cripps' Pink' apple color development and fruit ripening, and whether these effects are influenced by the application of AVG preharvest or 1-MCP postharvest.

Method and materials

Plant material and site description. The trial was conducted on the commercial farm Applegarth (34° 8' 11.5" S 19° 1' 49.4" E; 282 m. a. s. l.), in Elgin in the Western Cape, South Africa during the 2017/2018 season. 'Cripps' Pink' trees on M793 rootstock were selected. Trees were planted in 1995 at 4.0 m x 1.5 m, following a north-south row direction. A central leader training system was used with a micro-sprinkler irrigation system.

Treatments and experimental layout. The trial consisted of eight treatments and nine replicates in a randomized complete block design, as summarized in Table 1. Two products were evaluated preharvest, viz. ACC (Valent BioSciences Corporation, Libertyville, Illinois, USA) and AVG (ReTain®, Valent BioSciences Corporation, Libertyville, Illinois, USA) and 1-MCP postharvest (SmartFresh™, Agrofresh Solutions, Philadelphia, PA USA). Foliar treatments were applied either two weeks or one week before commercial harvest, on dates as presented in Table 2, using a motorized knapsack sprayer (STIHL, Pietermaritzburg, South Africa). Each tree was sprayed under favorable drying conditions, with air temperatures between 18 °C and 20 °C (Fig. 1), for approximately 30 seconds per side, equating to 1000 L of solution per ha. To prevent the effect of product drift, at least one tree was kept between treated trees as well as a buffer row between treatment rows. Within 24 hours of harvest, one treatment was taken to the laboratory at ExperiCo (Stellenbosch, Western Cape, South Africa) for a commercial rate 1-MCP application in a 0.7 m^3 airtight container for 24 hours (. Harvest dates are summarized in Table 2.

Data collection. At each harvest, all fruit complying with Pink Lady™ standards (blush coverage >40%) (Anon., 2020) were picked and weighed. To determine total yield per tree, the fruit weight from each harvest was taken and added together. Fruit drop was recorded at each harvest by counting fruit on the ground below the tree. A sample of 50 apples was collected at random at the first two harvests and immediately taken to the laboratory at Stellenbosch University for quality and maturity evaluations. At third harvest, no samples were taken due to laboratory capacity. All fruit samples were individually weighed, and color was evaluated by scoring the percentage blush coverage of fruit and scoring the blush color intensity of each fruit according to the Pink Lady™ color chart (Fig. 2). A sub-sample of 25 fruit was packed in commercial M4 cartons with plastic liner and placed in regular atmosphere (RA) storage at -0.5 °C for 12 weeks. The subsample of one treatment was treated as described above at ExperiCo (Stellenbosch, Western Cape, South Africa) within 24 hours of harvest, as specified in Table 1, and then placed into RA storage at -0.5 °C for 12 weeks. Fruit quality and maturity indexing parameters were evaluated on the remaining 25 fruit. Ground color of fruit was scored using the UNIFRUCO color chart for apples and pears (Fig. 3). Greasiness of fruit was scored on a scale of 0 to 3, 0 indicating no signs of greasiness, 1 slight greasiness, 2 moderate greasiness and 3 severe greasiness. A GÜSS texture analyzer (Güss electronic model GS 20, Strand, South Africa) fitted with an 11 mm probe was used to determine fruit firmness on parred cheeks. Starch breakdown of fruit at harvest was determined using an iodine solution and scored according to the UNIFRUCO starch conversion chart (Fig. 4). A digital refractometer (Model PR 32-α, Atago Co., Ltd., Tokyo, Japan) was used to determine total soluble solids (TSS) concentration of fruit at harvest.

Following the 12 weeks of RA storage, fruit ground color and greasiness of samples were evaluated. Shelf-life was then simulated by leaving samples at room temperature (±20 ºC) for seven days. Ground color and fruit greasiness were re-evaluated after shelf-life, as well as firmness, starch breakdown and TSS in the same manner as described for fruit at harvest.

Statistical analysis. The data were analyzed using SAS Enterprise guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA) using the linear model procedure. The Least Significant Difference (LSD) was determined using the pairwise t-test when treatment effects were significant ($P<0.05$).

Results and Discussion

Fruit color. ACC at 200 μL·L-1 two weeks before harvest (wbh) stimulated a significant increase in combined percentage blush coverage of ‘Cripps’ Pink’ apples weighted over the first two harvests. This was also the case for 200 µL・L-1 ACC in combination with the postharvest 1-MCP treatment (Table 3). These two treatments are the same at the point of harvest and therefore should not differ. The ACC applied 1 wbh did not differ significantly from either treatment mentioned before or the untreated control. Fruit treated with AVG alone developed significantly less blush color compared to all other treatments, irrespective of application timing. Both treatments combining AVG and ACC displayed lower blush coverage than fruit treated only with ACC, but a greater percentage blush coverage than fruit treated with AVG only and was not significantly different to the control. The same effect was observed in the combined percentage of fruit marketable as Pink Lady™ apples weighted over the first two harvests, but no significant differences were found in the percentage 'Cripps' Pink' and 3rd Class fruit (Table 3). The increase in red color was expected, since ACC is a precursor of ethylene, and the increase in red color is probably a response to upregulated ethylene synthesis due to the ACC application (Adams and Yang, 1981). The inverse is true for AVG application, as is expected, since AVG competitively inhibits ACS and thereby suppresses ethylene synthesis (Çetinbaş et al., 2012). Therefore, applying AVG before or after ACC partially reduced the ACC efficacy to advance maturity and thereby enhance red color development. However, it is important to note that combined percentages where calculated for two of the three harvests only, and given the varying percentages of yield harvested at the first two harvests for respective treatments (Table 3), comparisons between treatments should be interpreted with caution.

Although none of the treatments affected average percentage blush coverage significantly at the first harvest compared to the untreated control, fruit treated with 200 µL・L-1 ACC 2 wbh had significantly more blush coverage than AVG treatments both 1 and 2 wbh, as well as AVG applied 2 wbh in combination with ACC 1 wbh. The latter did not differ significantly from each other (Table 4). The ACC applied 1 wbh, as well as 2 wbh with AVG 1 wbh, did not differ significantly from any other treatment or the untreated control. There was no significant difference in the percentage fruit classified as Pink Lady™, 'Cripps' Pink' or third-class fruit at the first harvest, irrespective of treatment (Table 4). Fruit treated with ACC 2 wbh, but without postharvest 1-MCP, had more intense red color at first harvest than all other treatments and the untreated control (Table 4). Although the percentage blush coverage at the first harvest was not as clearly affected by the PGR as in the combined harvest data in Table 3, the effect on color intensity points to the same effect of ACC on color development. None of the remaining treatments differed significantly from the control in terms of red color intensity at first harvest. Both AVG applications on their own resulted in significantly lower red color intensity than ACC applied 2 wbh, 1 wbh, in combination with AVG 1 wbh as well as with postharvest 1-MCP. The same treatments did not have red color intensity significantly different to AVG applied 2 wbh with ACC 1 wbh at first harvest (Table 4). As mentioned before, AVG competitively inhibits ACS, thus slowing fruit ripening and thus color development (Doerflinger et al., 2019).

At the second harvest, fruit treated with AVG 1 wbh and a combination of ACC 2 wbh and AVG 1 wbh had significantly lower percentage blush coverage than untreated control fruit (Table 5). The percentage blush of fruit treated with ACC 2 wbh, AVG 2 and 1 wbh, AVG 2 wbh with ACC 1 wbh, as well as preharvest ACC treatment with postharvest 1-MCP treatment did not differ significantly from each other. However, this was not reflected in the percentage of fruit qualifying as Pink Lady™, which did not differ significantly among treatments. The percentage of fruit classified as 'Cripps' Pink' treated with AVG 1 wbh and ACC 2 wbh followed by AVG 1 wbh was significantly more than the untreated control. There were no significant differences among treatments or the control regarding the percentage of fruit qualifying as third-class at second harvest (Table 5). A significant decrease in red color intensity at second harvest was observed in fruit treated with AVG 1 wbh, as well as in combination with ACC 2 wbh compared to untreated control fruit (Table 5). Whale et al. (2008) in Australia treated 'Cripps' Pink' apple trees with ethephon (280 $g \cdot ha^{-1}$), AVG (125 $g \cdot ha^{-1}$) and a combination of the two, 5 wbh, and noted a significant increase in percentage red blush. They found an increase of 30% compared to the untreated control with ethephon alone, 12% with AVG alone and 23% with the combination of AVG applied 5 wbh and ethephon 18 days later. The authors also described an increase in the anthocyanin concentration in the peel of ethephon treated fruit as well as fruit treated with both compounds, whereas anthocyanin accumulation was unaffected by AVG application, resulting in anthocyanin concentration similar to that of control fruit. This concurs with the findings of our trial, whereby the percentage blush weighted over the first two harvests was increased by ACC treatment and decreased by AVG application, relative to the control. Silverman et al. (2004), on the other hand, reported that although AVG (125 $g \cdot ha^{-1}$) decreased ethylene synthesis over six weeks after application on 'Red Chief' apples, there was no effect on anthocyanin concentration in the fruit peel but this cultivar is a full dark red compared to the bicolor 'Cripps' Pink' that Whale et al. (2008) studied.

Yield. The total yield per tree was significantly increased by the AVG treatment 2 wbh compared to the untreated control, but did not differ significantly from the AVG application 1 wbh or ACC 2 wbh followed by AVG 1 wbh, although the latter did not differ significantly from the control (Table 6). Total yield was not affected by ACC treatment alone, or in combination with postharvest 1-MCP treatment compared to the control. AVG application 2 wbh followed by ACC 1 wbh decreased the total yield per tree, but not significantly so compared to the control. Therefore, none of the treatments differed significantly in yield compared to the control, which was expected. Significantly more fruit had dropped by the first harvest in response to the ACC treatment 2 wbh and ACC in combination with postharvest 1-MCP compared to the control (which at this stage is the same treatment) (Table 6). Fruit drop for all other treatments did not differ significantly from the control at first harvest and there were no statistical differences in fruit drop for either harvest two or three, regardless of treatment. Yildiz et al. (2012) reported that AVG reduced fruit drop when applied at 150 $mg \cdot L^{-1}$ 4 wbh to 'Red Chief' apples, compared to control fruit. In this trial, 125 $mg \cdot L^{-1}$ AVG did not reduce fruit drop relative to the control, but it was applied 2 wbh and not 4 wbh. AVG is registered in South Africa at 125 $mg \cdot L^{-1}$ 4 wbh and when applied at this time could reduce fruit drop (Philagro SA). In contrast, Petri et al. (2006) reported that fruit drop was better controlled in 'Gala' fruit when AVG was applied closer to harvest, regardless of the concentration, but did not have the same effect on 'Fuji' apples. The effect of AVG application timing on different apple cultivars could be an interesting point of study in the future. The increase in fruit drop induced by ACC 2 wbh was not reflected in total yield, and yield differences between treatments therefore cannot be explained by fruit drop. It is clear also that the fruit drop was a relatively fast response to the ACC treatment 2 wbh, as further fruit drop during the harvest period was not affected.

At first harvest, the greatest percentage fruit came from treatments where ACC was applied 2 wbh (Table 7). ACC alone did not differ from the treatment where it was combined with postharvest 1-MCP as expected, and the latter did not differ significantly from the combination of ACC followed by AVG 1 wbh. The same combination did not differ significantly from ACC applied 1 wbh, the control, AVG and ACC 1 wbh, or AVG applied 1 wbh. The lowest proportion of fruit at first harvest was harvested from trees treated with AVG 2 wbh, which also did not differ from the control. There were no significant differences in yield distribution at the second harvest. At the third harvest, a similar but opposite yield distribution was observed to the first harvest. Fruit treated with AVG alone at 1 wbh and 2 wbh displayed the greatest percentage fruit harvested at third harvest with 39.1% and 38.1%, respectively. This delay in harvest was expected, since AVG inhibits ethylene synthesis, and was the desired effect of AVG application. AVG is thus utilized as a harvest management tool. Petri et al. (2006), who also investigated the effect of varying concentrations of AVG on delaying fruit ripening on ‘Gala’ and ‘Fuji’ apples in Brazil, reported a delay in the start of ‘Gala’ harvest by 10 days from the control. The end of harvest in the same trial was 16 days later than the control, thus indicating a shift in the harvest window. The harvest percentage at the third harvest of fruit from AVG application 2 wbh in combination with ACC, the untreated control, ACC 2 wbh with AVG 1 wbh and ACC application 1 wbh, did not differ significantly from one another, but was significantly lower than AVG treatments alone (Table 7). Evidently, the combination of ACC and AVG treatment results in an intermediate effect in harvest management. It is interesting that it did not matter whether the AVG was applied 2 wbh before ACC or after the ACC application. ACC applied 2 wbh and ACC in combination with postharvest 1-MCP had only 8.8% and 6.7% of total yield harvested at third harvest, respectively, and did not differ significantly from each other or ACC applied 1 wbh (Table 7), indicating that ACC advanced the harvest, which is correlated to advanced fruit ripening caused by an upregulation of ethylene biosynthesis (Larrigaudiere et al., 1996).

Fruit quality and maturity at harvest. There were no significant differences in average fruit weight for either harvest, irrespective of treatment (Table 8). Treatments were only applied one or two weeks before harvest commenced, and even though AVG delayed maturity, all samples were taken simultaneously, thus not allowing fruit size differences to occur. The ground color of fruit treated with ACC 2 wbh was significantly yellower than the ACC with AVG, the untreated control, and both AVG-only applications (Table 9). Fruit treated exclusively with AVG displayed the greenest ground color compared to the control as well as all other treatments except AVG 2 wbh with ACC 1 wbh (Table 9). Yellower ground colour in fruit indicate advanced maturity and is reflected in the percentage starch breakdown observed at harvest. A significant increase in percentage starch breakdown was seen in fruit treated with ACC (including ACC with postharvest 1-MCP, ACC with AVG 1 wbh, ACC 2 wbh and ACC 1 wbh) compared to the control. Stern et al. (2010) similarly reported that 50 $mg \cdot L^{-1}$ 2, 4-DP, a synthetic auxin stimulating ethylene synthesis, applied to ‘Cripps’ Pink’ at different times between 45 and 90 days after full bloom caused an increase in the percentage starch degradation at harvest. Firmness was, however, unaffected in the same trial. Percentage starch breakdown was not significantly different compared to the control when AVG was applied 2 wbh followed by ACC 1 wbh , whereas the starch breakdown of fruit treated only with AVG at both 1 wbh and 2 wbh was significantly lower than all other treatments, but not the control. This finding is supported by Silverman et al. (2004) who reported reduced starch degradation in 'Red Chief' apples when treated with AVG 4 wbh. The increase in percentage starch breakdown confirms the advanced maturity of ACC treated fruit as indicated by the yellower ground color, and vice versa for AVG treated fruit. The same trend, however, is not reflected in average fruit firmness or TSS, where no significant differences were noted. Although TSS is not a good indicator of fruit maturity, a decrease in fruit firmness was expected after ACC application, whereas one would expect fruit firmness to be maintained after AVG application (Table 9). Greasiness incidence indicates advanced maturity especially after storage, and notably so in Pink Lady™ apples (Anon., 2019). In our study, there was an increase in slight greasiness incidence of fruit treated with ACC on its own 1 wbh and 2 wbh, compared to the untreated control, even though greasiness incidence was low, as expected immediately at harvest. No other treatments differed significantly from the untreated control (Table 9).

At the second harvest the same trend was observed for ground color as at the first harvest for both ACC and AVG treated apples. Fruit treated with ACC 2 wbh had significantly yellower ground color than all other treatments (Table 10). Ground color of fruit treated with ACC 1 wbh as well as 2wbh with 1-MCP postharvest was statistically less yellow than the before mentioned treatment, and yellower than all other treatments, which did not differ significantly from each other. Starch breakdown at second harvest was significantly advanced by all ACC treatments and retarded by treatments with AVG on its own, although all treatments were harvested at post optimum starch breakdown (>30%). In contrast to the first harvest, the percentage starch breakdown of fruit treated with AVG either 2 wbh or 1 wbh differed significantly from the untreated control, at 43% and 47%, respectively compared to the 58% of the control fruit. Fruit firmness of all treatments were similar at the second harvest compared to the control, although unlike at the first harvest, slight differences were observed among treatments, none of which indicate a conclusive trend. There were no significant differences in average fruit TSS at the second harvest irrespective of treatment. There was more greasiness incidence observed at the second harvest than at the first harvest. The slight greasiness incidence was significantly more in fruit treated with ACC 2 wbh than fruit from all other treatments at the second harvest. Fruit treated with AVG either 2 or 1 wbh displayed the least slight greasiness incidence at second harvest but was not statistically different from the untreated control or AVG 2 wbh with ACC 1 wbh. Furthermore, slight greasiness of fruit treated with ACC 1 wbh, 2wbh followed by AVG 1 wbh and in combination with 1-MCP was statistically similar to untreated control fruit. As mentioned before, greasiness incidence is associated with fruit maturity and again illustrated in fruit from the second harvest.

Fruit quality and maturity after storage and shelf-life. Surprisingly, after 12 weeks storage at -0.5 ºC in RA, first harvest fruit ground color was most advanced in fruit treated with the combination of ACC 2 wbh and postharvest 1-MCP (Table 11). This phenomenon cannot be explained. All other treatments had similar fruit ground color to the untreated control. All first harvest fruit treated with AVG were greener after storage than the two treatments receiving only ACC. Second harvest fruit treated only with ACC and fruit treated with ACC followed by 1-MCP postharvest were the yellowest, followed by untreated control fruit, a trend similar to that of the first harvest. The ground color of fruit treated with AVG as well as both combinations of ACC and AVG were the same and the greenest. Slight greasiness was observed after storage, but levels ranged between 2.2 and 0% in first harvest and 0.9 and 0% in second harvest fruit and was not negatively affected by any treatment (Table 11).

After seven days of simulated shelf-life (20°C), the ground color of first harvest fruit was yellowest in fruit treated only with ACC and in the untreated control (Table 12). Yellower ground color - indicates advanced fruit maturity, as is expected with ACC treatment (Whale and Singh, 2007). Ground color of fruit treated with AVG 2 wbh and in combination with ACC, as well as ACC with 1-MCP, were significantly greener than untreated control fruit after simulated shelf-life. Since the ground color of postharvest 1-MCP treated fruit evaluated after shelf-life does not reflect the unexpected result observed after storage, it may be possible that human error could be to blame for inconsistent scoring post storage. The starch breakdown of all fruit was 100%, except fruit treated with post-harvest 1-MCP, which had significantly lower starch breakdown (96%) which is consistent with the less advanced maturity expected following postharvest 1-MCP treatment (Crouch et al., 2005). This is also reflected in fruit firmness after shelf-life that was also significantly greater in fruit treated with postharvest 1-MCP compared to all other treatments. After shelf-life, first harvest fruit of all other treatments displayed firmness similar to the control, except for ACC 2 wbh with AVG 1 wbh fruit, which were significantly firmer than control fruit. The average fruit TSS of all treatments was statistically similar to the control fruit, except fruit treated with AVG 2 wbh, which had a significantly higher TSS (Table 12). Slight greasiness was more prevalent than immediately after storage and was highest when fruit had been treated with ACC, especially 2 wbh compared to all other treatments (more than double that of ACC 1 wbh) and the untreated control, once again indicating that ACC advanced fruit maturity and that timing of ACC application is an important factor to consider.

After simulated shelf-life, the ground color of second harvest fruit treated with ACC 2 wbh was significantly yellower than all other treatments (Table 13). Fruit from treatments receiving AVG application 2 wbh, 1 wbh, in combination with ACC 1 wbh as well as ACC 2 wbh and postharvest 1-MCP had greener ground colour than all other treatments and the untreated control. The ground color of fruit treated with ACC 1 wbh and ACC 2 wbh with AVG 1 wbh was statistically similar to control fruit from the second harvest when evaluated after shelf-life. The findings of ground color at second harvest reiterate the efficacy of AVG and 1-MCP to delay fruit ripening through inhibition of ethylene synthesis or its action respectively, and ACC to advance fruit ripening by stimulating ethylene biosynthesis. The starch breakdown of all fruit at second harvest after shelf-life was 100% irrespective of treatment (data not shown). Fruit treated with ACC 2 wbh and 1-MCP after the second harvest were significantly firmer than fruit from all other treatments and the untreated control post shelf-life, as was the case at first harvest. The fruit firmness of all other treatments were statistically similar to control fruit, except fruit treated with AVG 2 wbh and ACC 1 wbh, which had significantly firmer fruit than the untreated control, but less firm than ACC and 1-MCP treated fruit. After shelf-life, the average TSS of second harvest fruit treated with AVG, and AVG in combination with ACC and the ACC/1-MCP treated fruit was significantly higher compared to the untreated control, whereas TSS of fruit from all other treatments did not differ significantly from the untreated control (Table 13). The percentage fruit with slight greasiness was significantly more in fruit treated with ACC 2 wbh than all other treatments, all of which were statistically similar to untreated control fruit. Greasiness incidence post shelf-life at second harvest generally reflected the same trend as seen at first harvest post shelf-life, where an increase in greasiness was observed in fruit treated with ACC, indicating advanced fruit maturity and less greasiness incidence in AVG and 1-MCP treated fruit, indicating that the ripening process was delayed in these fruit.

Conclusion

It is evident from this trial that ACC increased the blush percentage of fruit combined over two harvest dates by advancing fruit maturity, while AVG delayed red color development of ‘Cripps’ Pink’ apples. This subsequently resulted in an increase in the percentage fruit classed as Pink Lady™ for ACC-treated fruit and a decrease when treated with AVG when fruit were harvested on the same date, but not at the same maturity. Although generally, but not always significantly, a greater percentage Pink Lady™, and therefore export-grade fruit, was harvested with ACC application, it would not be recommended to export these fruit at the high levels of starch degradation at harvest as shown in this trial. In both instances, the combination of ACC and AVG had an effect similar to the control, as ethylene synthesis was probably accelerated by ACC application and delayed with AVG application. AVG also delayed fruit maturation, as reflected by starch breakdown at harvest, which was consistently lower than the control and significantly so at second harvest. When used in combination with ACC, the same effect was not observed. This could be due to application timing, since standard protocol for applying AVG is 4 wbh, not 2 wbh as in our study. Results should, however, be interpreted with caution, since the percentage starch breakdown of fruit from all treatments exceeded the 30% optimum at first and second harvest, but especially so in ACC-treated fruit. In order to make concrete conclusions, all fruit would have had to be harvested at optimum starch breakdown and thus maturity. Future research should be dedicated to establishing the optimum time of application of AVG in combination with ACC should this prove to be an economically beneficial practice for increasing red color of fruit without negatively affecting fruit maturity.

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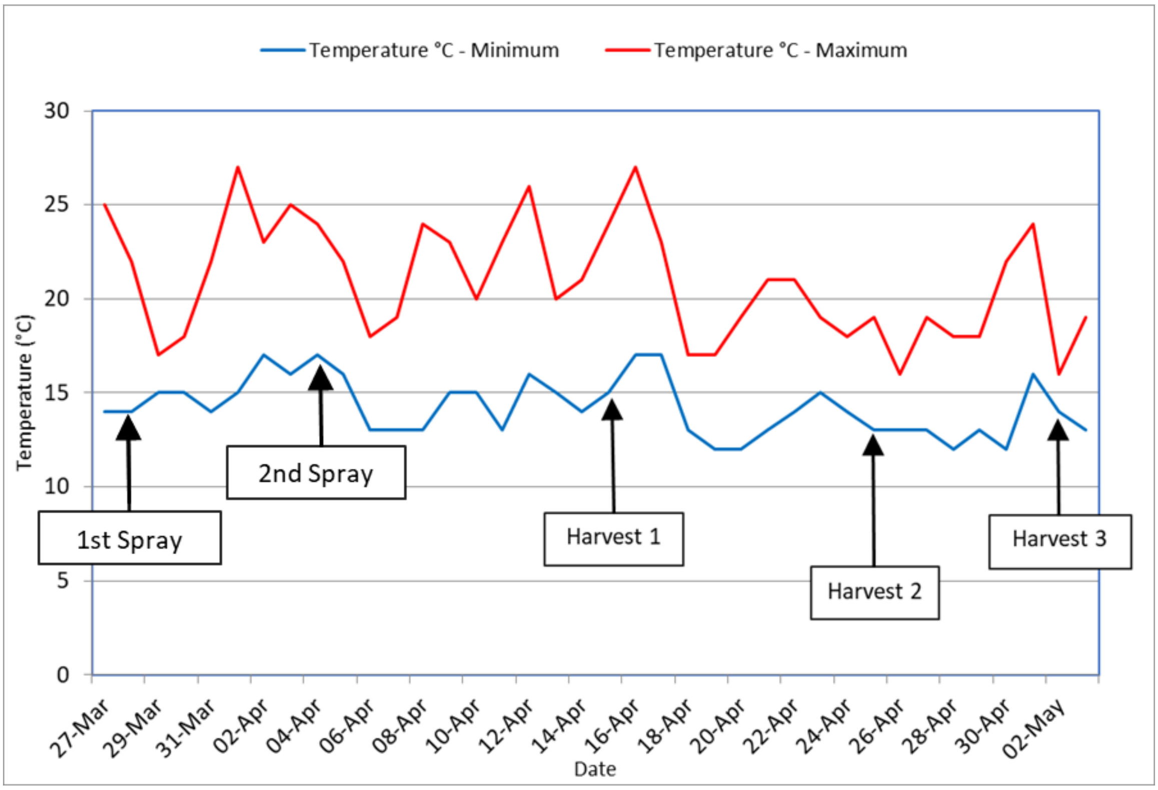

Fig. 1. Weather data at the 'Cripps' Prink' trial site in 2018 at Applegarth, Grabouw, South Africa

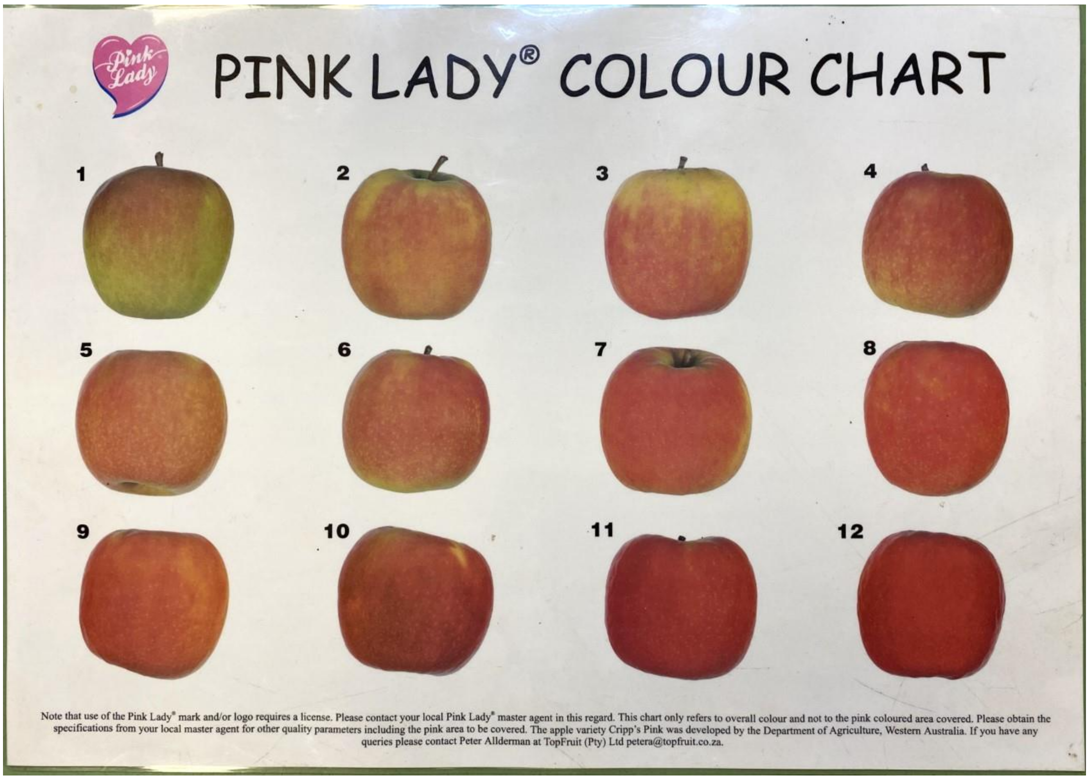

Fig. 2. Pink Lady™ color chart used to determine the blush color intensity of ‘Cripps’ Pink’ apples.

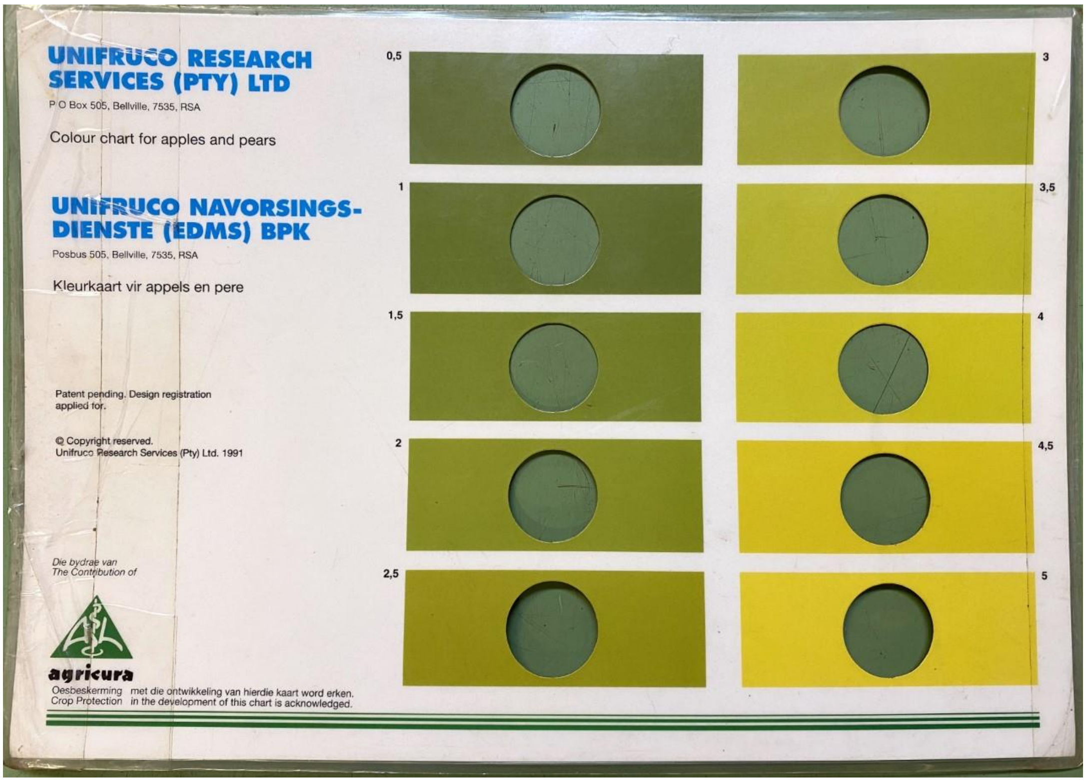

Fig. 3. UNIFRUCO color chart used to determine ground color of apples.

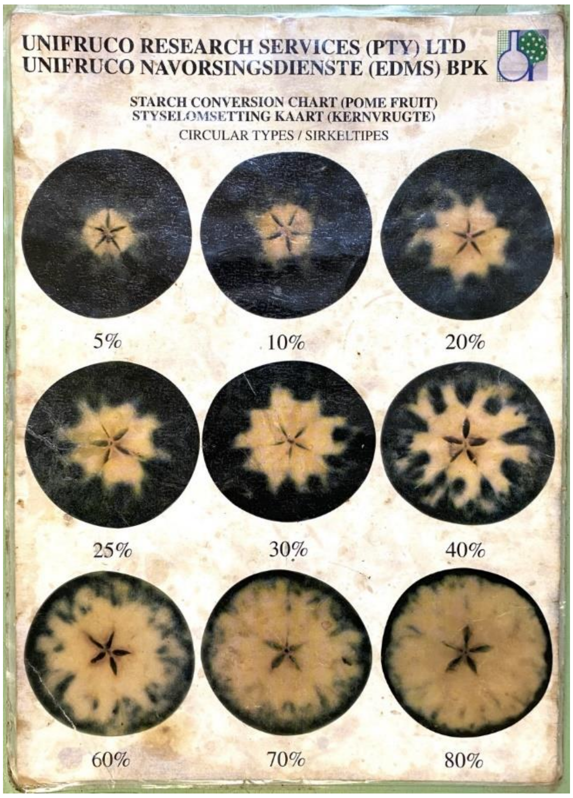

Fig. 4. UNIFRUCO chart used to determine starch conversion of apples.

Table 1. Treatment summary for trials done on Applegarth in 2018 with 1-aminocyclopropane-1-carboxylic acid (ACC), aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP) at one and two weeks before harvest (wbh) on 'Cripps' Pink' apples.

Orchard treatment	Post-harvest treatment
Untreated control	None
ACC* (200 µL·L-1) at 2 wbh	None
ACC* (200 µL·L-1) at 1 wbh	None
AVG* (125 mg·L^{-1}) at 2 wbh	None
AVG* (125 mg·L^{-1}) at 1 wbh	None
ACC* (200 µL·L-1) at 2 wbh and AVG* (125 mg·L^{-1}) at 1 wbh	None
AVG* (125 mg·L^{-1}) at 2 wbh and ACC* (200 µL·L-1) at 1 wbh	None
ACC* (200 µL·L-1) at 2 wbh	1-MCP

*ACC applied with surfactant: Villa 51 @ 6 µL·L^{-1}; AVG applied with surfactant: Breakthru @ 50 µL·L^{-1}

Table 2. Summary of the dates of treatment applications and harvest for 'Cripps' Pink' apples at Applegarth, Elgin (2018). *1-aminocyclopropane-1-carboxylic acid (ACC), aminoethoxyvinylglycine (AVG), 1-methylcyclopropene (1-MCP), maturity indexing (MI)

Application and harvest dates	
Application of ACC* and AVG* 2 wbh	28 March 2018
Application of ACC and AVG 1 wbh	4 April 2018
First commercial harvest (Harvest 1)	16 April 2018
Application of 1-MCP*	17 April 2018
Second commercial harvest (Harvest 2)	25 April 2018
Application of 1-MCP	26 April 2018
Third commercial harvest (Harvest 3)	2 May 2018
Post-storage MI* (harvest 1 samples)	9 July 2018
Post-storage MI (harvest 2 samples)	16 July 2018

Table 3. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on the percentage blush and color distribution combined over the first two harvests for 'Cripps' Pink' apples at Applegarth, Elgin (2018).

Treatment	Combined percentage blush coverage over first two harvests		Combined percentage Pink Lady™* over first two harvests		Combined percentage 'Cripps' Pink'* over first two harvests		Combined percentage 3rd class* fruit over first two harvests		Percentage of yield harvested at first two harvests
Control	38.8	bc	63.6	bc	11.3	ns	2.1	ns	77.0
ACC 2 wbh**	47.7	a	76.4	a	13.0		1.8		91.2
ACC 1 wbh	43.2	ab	71.0	ab	13.3		2.6		86.9
AVG 2 wbh	28.4	d	48.1	d	10.3		3.6		62.0
AVG 1 wbh	28.1	d	47.6	d	11.3		2.0		60.9
ACC + AVG	36.8	c	59.2	c	16.7		3.1		79.0
AVG + ACC	35.3	c	58.8	c	14.0		3.0		75.8
ACC + 1-MCP	47.1	a	75.3	a	12.9		5.1		93.3
Significance level	***<.0001***		***<.0001***		*0.4078*		*0.0774*		-
LSD 5%	*6.1*		*10.0*		-		-		-

*Blush >40% = Pink Lady™; blush 20-40% = 'Cripps' Pink'; blush <20% = 3rd class

**2 weeks before harvest

Table 4. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on red color development of 'Cripps' Pink' apples at the first harvest at Applegarth, Elgin (2018).

Treatment	Percentage blush coverage		Percentage Pink Lady™*		Percentage 'Cripps' Pink'*		Percentage 3rd class* fruit		Red color intensity**	
Control	51.9	abc	87.5	ns	11.6	ns	0.9	ns	7.32	bcd
ACC 2 wbh***	55.3	a	91.3		8.2		0.5		8.50	a
ACC 1 wbh	51.9	abc	87.3		12.5		0.2		7.91	b
AVG 2 wbh	48.0	c	83.0		14.4		2.5		6.81	cd
AVG 1 wbh	49.8	c	87.1		12.1		0.9		6.78	d
ACC + AVG	52.2	abc	87.2		10.5		2.2		7.39	bc
AVG + ACC	50.4	bc	86.4		12.5		1.1		6.86	cd
ACC + 1-MCP	54.4	ab	88.0		9.1		2.9		7.73	b
Significance level	***0.0369***		*0.6535*		*0.6228*		*0.0701*		***<.0001***	
LSD 5%	*4.4*		-		-		-		*0.59*	

*Blush >40% = Pink Lady™; blush 20-40% = 'Cripps' Pink'; blush <20% = 3rd class

**Scored from 1 to 12 with 1 no color and 12 intense color (Fig. 2)

***2 weeks before harvest

Table 5. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on red color development of 'Cripps' Pink' apples at the second harvest at Applegarth, Elgin (2018).

Treatment	Percentage blush coverage		Percentage Pink Lady™*		Percentage 'Cripps' Pink'*		Percentage 3rd class* fruit		Red color intensity**	
Control	47.4	a	75.6	ns	19.2	c	5.1	ns	6.05	a
ACC 2 wbh***	45.6	ab	69.2		25.3	bc	5.6		5.75	ab
ACC 1 wbh	48.0	a	75.8		18.8	c	5.4		6.03	a
AVG 2 wbh	44.0	abc	71.7		19.5	c	8.8		5.56	abc
AVG 1 wbh	41.1	bc	65.4		28.1	ab	6.5		5.25	bc
ACC + AVG	38.8	c	58.6		36.2	a	5.1		4.93	c
AVG + ACC	43.2	abc	70.0		23.4	bc	6.5		5.52	abc
ACC + 1-MCP	43.8	abc	67.3		13.1	c	9.6		5.56	abc
Significance level	***0.0387***		*0.0674*		***0.0012***		*0.5413*		***0.0418***	
LSD 5%	*5.7*		-		*8.2*		-		*0.71*	

*Blush >40% = Pink Lady™; blush 20-40% = 'Cripps' Pink'; blush <20% = 3rd class
**Scored from 1 to 12 with 1 no color and 12 intense color (Fig. 2)
***2 weeks before harvest

Table 6. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on total yield and fruit drop of 'Cripps' Pink' apples at Applegarth, Elgin (2018).

Treatment	Total yield per tree (kg)		Number of fruit picked up below tree harvest 1		Number of fruit picked up below tree harvest 2		Number of fruit picked up below tree harvest 3	
Control	64.0	bc	18	c	7	ns	4	ns
ACC 2 wbh*	61.0	bc	34	a	7		2	
ACC 1 wbh	62.1	bc	19	c	9		2	
AVG 2 wbh	85.2	a	17	c	8		5	
AVG 1 wbh	70.3	abc	17	c	8		6	
ACC + AVG	74.7	ab	24	bc	6		2	
AVG + ACC	56.5	c	24	bc	8		3	
ACC + 1-MCP	65.4	bc	30	ab	7		2	
Significance level	***0.0400***		***0.0013***		*0.7972*		*0.0801*	
LSD 5%	*17.0*		*9.0*		-		-	

*2 weeks before harvest

Table 7. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on yield distribution of 'Cripps' Pink' apples at Applegarth, Elgin (2018).

Treatment	Percentage harvest 1		Percentage harvest 2		Percentage harvest 3	
Control	41.3	cd	35.6	ns	23.1	b
ACC 2 wbh*	62.5	a	28.7		8.8	c
ACC 1 wbh	44.6	c	42.3		13.1	bc
AVG 2 wbh	31.0	d	31.0		38.1	a
AVG 1 wbh	35.5	cd	25.4		39.1	a
ACC + AVG	46.5	bc	32.5		21.0	b
AVG + ACC	36.5	cd	39.3		24.2	b
ACC + 1-MCP	59.3	ab	34.0		6.7	c
Significance level	***<.0001***		*0.0934*		***<.0001***	
LSD 5%	*13.6*		-		*11.2*	

*2 weeks before harvest

Table 8. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on fruit weight of 'Cripps' Pink' apples at Applegarth Elgin (2018).

Treatment	Average fruit weight harvest 1 (g)		Average fruit weight harvest 2 (g)	
Control	134.87	ns	138.55	ns
ACC 2 wbh*	140.60		139.98	
ACC 1 wbh	142.40		140.37	
AVG 2 wbh	133.36		137.01	
AVG 1 wbh	138.01		140.33	
ACC + AVG	134.70		137.03	
AVG + ACC	136.31		139.81	
ACC + 1-MCP	134.94		135.72	
Significance level	*0.5796*		*0.9522*	
LSD 5%	-		-	

*2 weeks before harvest

Table 9. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on fruit quality and maturity of 'Cripps' Pink' apples at the first harvest at Applegarth, Elgin (2018).

Treatment	Ground color*		Average fruit percentage starch conversion		Average fruit firmness (kg)		Average fruit TSS (%)		Percentage fruit with slight greasiness	
Control	3.29	cd	50.18	cd	8.63	ns	13.73	ns	0.4	c
ACC 2 wbh**	3.48	a	68.22	ab	8.66		14.04		6.2	a
ACC 1 wbh	3.38	abc	63.56	ab	8.56		13.98		4.0	ab
AVG 2 wbh	3.18	e	44.51	d	8.69		14.06		1.3	bc
AVG 1 wbh	3.13	e	43.22	d	8.73		13.73		1.3	bc
ACC + AVG	3.34	bc	68.38	ab	8.68		13.99		1.8	bc
AVG + ACC	3.22	de	57.89	bc	8.83		13.89		0.0	c
ACC + 1-MCP	3.43	ab	70.73	a	8.79		14.10		2.7	bc
Significance level	***<.0001***		***<.0001***		*0.6161*		*0.7340*		***0.0181***	
LSD 5%	*0.11*		*11.16*		-		-		*3.5*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Fig. 3)
**2 weeks before harvest

Table 10. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on fruit quality and maturity of 'Cripps' Pink' apples at the second harvest at Applegarth, Elgin (2018).

Treatment	Ground color*		Average fruit percentage starch conversion		Average fruit firmness (kg)		Average fruit TSS (%)		Percentage fruit with slight greasiness	
Control	3.07	c	57.77	c	8.35	abc	13.89	ns	12.5	bc
ACC 2 wbh**	3.54	a	71.53	ab	8.32	bc	13.74		55.1	a
ACC 1 wbh	3.25	b	78.53	a	8.35	abc	13.76		24.0	b
AVG 2 wbh	3.01	c	42.77	d	8.55	a	14.08		5.0	c
AVG 1 wbh	3.01	c	47.16	d	8.44	ab	13.79		6.3	c
ACC + AVG	3.04	c	69.89	b	8.21	c	13.98		23.6	b
AVG + ACC	3.07	c	71.99	ab	8.26	bc	14.04		17.1	bc
ACC + 1-MCP	3.27	b	73.02	ab	8.47	ab	14.33		20.4	b
Significance level	***<.0001***		***<.0001***		***0.0463***		*0.1504*		***<.0001***	
LSD 5%	*0.12*		*8.53*		*0.21*		-		*13.2*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Fig. 3)
**2 weeks before harvest

Table 11. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on ground color and greasiness of ‘Cripps’ Pink’ apples after 12 weeks storage from the first and second harvest at Applegarth, Elgin (2018).

Treatment	Ground color*				Percentage fruit with slight greasiness			
	First harvest		Second harvest		First harvest		Second harvest	
Control	3.13	bc	3.19	b	1.9	ab	0.4	ns
ACC 2 wbh**	3.21	b	3.43	a	2.2	a	0.0	
ACC 1 wbh	3.19	b	3.39	a	0.9	abc	0.9	
AVG 2 wbh	3.00	c	3.00	c	0.0	c	0.0	
AVG 1 wbh	3.00	c	3.00	c	0.0	c	0.4	
ACC + AVG	3.02	c	3.00	c	0.9	abc	0.8	
AVG + ACC 200	3.02	c	3.00	c	0.4	bc	0.0	
ACC + 1-MCP	3.36	a	3.50	a	0.0	c	0.8	
Significance level	***<.0001***		***<.0001***		***0.0821***		*0.1308*	
LSD 5%	*0.14*		*0.16*		*1.8*		-	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Fig. 3)

**2 weeks before harvest

Table 12. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on fruit quality and maturity of ‘Cripps’ Pink’ apples after 12 weeks RA storage and seven days shelf-life from the first harvest at Applegarth, Elgin (2018).

Treatment	Ground color*		Average fruit percentage starch conversion		Average fruit firmness (kg)		Average fruit TSS (%)		Percentage fruit with slight greasiness	
Control	3.99	ab	100.0	a	6.54	cd	14.24	bc	2.8	c
ACC 2 wbh**	4.16	a	100.0	a	6.69	cd	14.21	bc	32.5	a
ACC 1 wbh	4.00	ab	100.0	a	6.44	d	13.99	c	11.7	b
AVG 2 wbh	3.72	cd	100.0	a	6.72	bcd	14.68	a	1.8	c
AVG 1 wbh	3.81	bc	100.0	a	6.61	cd	14.50	ab	1.4	c
ACC + AVG	3.54	d	100.0	a	7.00	b	14.40	abc	2.4	c
AVG + ACC	3.67	cd	100.0	a	6.72	bcd	14.39	abc	2.2	c
ACC + 1-MCP	3.58	d	96.1	b	8.66	a	14.61	ab	2.2	c
Significance level	***<.0001***		***<.0001***		***<.0001***		***0.0447***		***<.0001***	
LSD 5%	*0.20*		*1.6*		*0.28*		*0.43*		*8.6*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Fig. 3)

**2 weeks before harvest

Table 13. Effect of preharvest 1-aminocyclopropane-1-carboxylic acid (ACC) (200 µL·L-1) and aminoethoxyvinylglycine (AVG) (125 mg·L^{-1}) and postharvest 1-methylcyclopropene (1-MCP) on fruit quality and maturity of ‘Cripps’ Pink’ apples after 12 weeks RA storage and seven days shelf-life from the second harvest at Applegarth, Elgin (2018).

Treatment	Ground color*		Average fruit firmness (kg)		Average fruit TSS (%)		Percentage fruit with slight greasiness	
Control	3.92	b	6.49	cd	13.83	c	9.0	bc
ACC 2 wbh**	4.26	a	6.32	d	13.78	c	33.8	a
ACC 1 wbh	4.00	b	6.37	cd	14.07	bc	15.5	b
AVG 2 wbh	3.59	c	6.56	c	14.47	a	0.4	c
AVG 1 wbh	3.67	c	6.53	cd	14.07	bc	2.3	c
ACC + AVG	3.94	b	6.50	cd	14.41	ab	9.0	bc
AVG + ACC	3.67	c	6.85	b	14.13	abc	7.7	bc
ACC + 1-MCP	3.60	c	8.43	a	14.47	a	1.8	c
Significance level	***<.0001***		***<.0001***		***0.0010***		***<.0001***	
LSD 5%	*0.23*		*0.24*		*0.38*		*9.7*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Fig. 3)
**2 weeks before harvest

PAPER 2: The Use of Plant Growth Regulators to Improve Red Color Development in 'Fuji' and 'Cripps' Pink' Apples

Additional index words. Internal ethylene concentration, 1-aminocyclopropane-1-carboxylic acid, 1-methylcyclopropene

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Abstract.

Fruit red color coverage and intensity determines the market value of bi-color apple cultivars, such as 'Braeburn', 'Fuji' and 'Cripps' Pink'. Anthocyanin synthesis and therefore red color development is highly light- and temperature-dependent, which proves challenging in warmer Mediterranean-type climates such as the Western Cape, South Africa. Color development also occurs simultaneously with fruit ripening, a process that is highly ethylene sensitive. The aim of this study was, therefore, to determine the efficacy of a foliar application of 1-aminocyclopropane-1-carboxylic acid (ACC) (100 µL $\cdot$L^{-1} – 400 µL $\cdot$L^{-1}), the precursor to ethylene, two weeks before harvest to upregulate fruit ethylene synthesis and therefore red color development. This was followed by a postharvest treatment of 1-methylcyclopropene (1-MCP) to half the fruit samples in order to counteract possible negative fruit ripening effects from the ACC treatment. The study was carried out over two consecutive seasons on 'Cripps' Pink' and one season on 'Fuji' apples. A rapid increase in internal ethylene concentration of fruit after ACC application in both seasons indicated that ACC was taken up by the fruit and converted to ethylene. This resulted in a shift in harvest distribution for both cultivars, whereby a greater proportion of the total harvest came from the earliest harvest date. Since fruit are harvested according to a minimum of 40% red color for 'Cripps' Pink' and 50% for 'Fuji', market depending, one can infer that the shift in harvest distribution due to ACC application indicated that color development was stimulated by ACC treatment in both cultivars. Loss in fruit firmness, greater starch breakdown and more yellow ground color in both 'Cripps' Pink' and 'Fuji' apples, however, indicate that fruit maturity was advanced by treatment with ACC, especially at the high rate (400 µL$\cdot$L^{-1}). Postharvest 1-MCP treatment successfully prevented the loss of fruit firmness and maintained ground color for both cultivars. It is clear from this study that ACC has a rate specific effect on

color development and fruit maturity. However, when applied at 200 μL·L^{-1} followed by postharvest 1-MCP treatment, ACC showed potential for aiding in color development of bi-color apples without negatively affecting fruit maturity; but fruit should be harvested at optimum maturity.

The red color of bi-color apple cultivars allows these to be distinguishable in the market and is fundamental in grading standards for exports. Consumer preferences favor fruit with a more pronounced blush and intense red color, since this is associated with a higher antioxidant content and thus greater health benefits than fruit with a dull appearance (Hamadziripi et al., 2014; Thomson et al., 2018). Color development therefore holds substantial economic value, especially for producers who aim to export their fruit.

Red color development results from anthocyanin biosynthesis and accumulation, often coinciding with simultaneous degradation of chlorophyll in the fruit peel (Tijskens et al., 2011). The main anthocyanin responsible for red coloration in apples is cyanidin-3-galactoside (Idaein) (Saure, 1990). Red color development in apple fruit is influenced by genetic, environmental, and developmental factors as well as cultural practices (Saure, 1990). Exposure of fruit to light is critical to red color development as well as optimal photosynthetic activity to sustain plant and fruit growth (Shafiq et al., 2014). However, with correct planting strategies and pruning, light is potentially a non-limiting factor in the Mediterranean-type climate of South Africa (Musacchi and Serra, 2018). Warm temperatures are, however, detrimental to the temperature-dependent anthocyanin biosynthetic pathway, where increased temperatures hinder sufficient red color development (Honda and Moriya, 2018; Gouws and Steyn, 2014), a trend which is becoming increasingly problematic as global temperatures rise.

Since an increase in anthocyanin biosynthesis in apple coincides with ripening, ethylene plays a significant role in red color development. Exogenous preharvest application of ethylene as Ethephon (2-chloroethylphosphonic acid) successfully improved red color of ‘Fuji’ and ‘Cripps’ Pink’ apples (Li et al., 2001; Whale et al., 2008; Shafiq et al., 2014). However, advances in fruit maturity were observed in both cultivars, thus decreasing storability of fruit. 2-Chloroethylphosphonic acid (Ethephon) application also poses the risk of leaving an undesirable residue on fruit (European Food Safety Authority, 2008) for which the European market has a minimum residue level (MRL) of 0.8 mg·kg^{-1} on apples (European Commission, 2005). Ethylene synthesis can also be enhanced endogenously by applying 1-aminocyclopropane-1-carboxylic acid (ACC), which is a precursor for ethylene (Van de Poel and Van Der Straeten, 2014). Although fruit maturity will still be hastened, the risk of residue is eliminated. Currently, there are various strategies for halting the fruit ripening process. This can either be done with preharvest application of aminoethoxyvinylglycine (AVG) (Yildiz et al., 2012), which inhibits the activity of the rate limiting enzyme ACC synthase (ACS), or with pre- or postharvest application of 1-MCP which stops ethylene action by blocking ethylene receptor sites (MacLean et al., 2007). Doerflinger et al. (2019) showed that 1-MCP applied to 'Empire' and 'McIntosh' apples one week before harvest successfully maintained low levels of internal ethylene and greater fruit firmness than control fruit after storage, up to 168 days after harvest. They also noted that the effectiveness of pre-harvest 1-MCP treatment on maintaining fruit maturity is highly sensitive to application time.

The aim of this paper was to investigate the potential of a preharvest foliar application of ACC to increase red color development, in combination with a postharvest 1-MCP treatment to halt the ripening process.

Method and materials

Plant material and site description for the 2017/2018 season. The location of the trial was the commercial farm Applegarth (34° 8' 11.5" S 19° 1' 49.4" E; 282 m. a. s. l.) in Elgin in the Western Cape, South Africa. 'Cripps' Pink' trees on M793 rootstock were planted 4 m x 1.5 m in 1995 in a north-south row direction. A central leader training system was used with a micro-sprinkler irrigation system.

Treatments and experimental layout for the 2017/2018 season. Ten two-tree plot replications of five treatments of different rates of ACC (Valent BioSciences Corporation, Libertyville, Illinois 60048, USA) were applied two weeks before estimated harvest commencement in a randomized complete block design. Treatments are described in Table 1. Motorized knapsack sprayers (STIHL, Pietermaritzburg, South Africa) were used for the foliar applications, where each tree was sprayed for approximately 30 seconds per side at an equivalent of approximately 1000 L per ha. All applications were made under slow drying conditions with air temperatures between 18 °C and 20 °C (Fig. 1). Very light rainfall was experienced the day following application; however, favorable conditions for at least ten hours after application ensured uptake of the product. At least one untreated tree was left between treatments as well as a buffer row between treated rows to prevent the effect of product drift. After harvest, fruit samples were either treated with 1-MCP (SmartFresh™; AgroFresh Solutions Inc., Philadelphia, Pennsylvania, USA) or left untreated, thereby creating a split-plot design. Fruit treated with 1-MCP were placed in 0.7 m^3 airtight containers where they were treated with commercially registered rate of 1-MCP for 24 hours at the ExperiCo (Agri-Research Solutions) laboratory (Stellenbosch, Western Cape, South Africa). In total, 75 fruit samples were taken per plot, 25 of which were taken for immediate maturity indexing, 25 treated with 1-MCP and placed in RA storage for 12 weeks and the remaining 25 were placed in RA storage for 12 weeks without 1-MCP treatment. Dates of application and harvest are summarized in Table 2.

Plant material and site description for the 2018/2019 season. During the 2018/2019 season, two trials were conducted on bi-color apples, one on 'Cripps' Pink' and the other on 'Fuji Brak' (Kiku). 'Cripps' Pink' trees on Lourensford (34° 3' 58.9" E, 18° 53' 22.4" S; 13 m. a. s. l.) near Somerset West in the Western Cape, South Africa were selected. The trees on M.793 rootstock were planted at 4 m x 1.5 m in 1998 in a north-south row direction. The 'Fuji' trial was conducted on Graymead (34° 1' 32.1" S, 19˚ 7' 49.3" E; 331 m. a. s. l.) in the Vyeboom region in the Western Cape, South Africa. Trees on M. 793 were planted in 2009 at 4 x 2 m, following a north-south row direction.

Treatments and experimental layout for the 2018/2019 season. In both trials a randomized complete block design was used with ten two-tree plot replications of five treatments as described for 2017/2018 (Table 1). Foliar treatments for both trials were applied with a motorized knapsack sprayer as described for the 2017/2018 season. Applications were made between air temperatures of 15 °C and 20 °C under slow drying conditions (Fig. 2 and 3). Dates of application and harvests are summarized in Table 3.

Data collection. During both seasons, ten 'Cripps' Pink' apples were sampled for internal ethylene concentration (IEC) evaluations before spray application, as well as at intervals after application as summarized in Table 4 and Table 5 for the two seasons, respectively. In the 2017/2018 season, fruit from control trees as well as 400 $\mu L \cdot L^{-1}$ ACC treated trees were sampled, whereas additional samples were taken from 200 $\mu L \cdot L^{-1}$ ACC treated trees in the 2018/2019 season. Fruit samples were immediately taken to the laboratory at Stellenbosch University to be analyzed. Fruit samples were placed in a glass bell jar, two at a time, and submerged in de-gassed water. A vacuum pump attached to the bell jar was used for fruit internal gas extraction, causing displacement of fruit internal gas with water. After visible air collected at the top of the jar, a 10 mL gas sample was extracted using an airtight syringe (VICI Precision sampling, Baton Rouge, LA) via a septum. Within 24 hours of sampling, a 5 mL headspace sample was assessed on a gas chromatograph (GC) (Model N6980, Agilent Inc., Wilmington, U.S.A.) fitted with a PorapakQ and a Molsieve packed column, flame ionization and thermal conductivity detectors. The oven temperature was held constant at 80 °C. Internal ethylene was expressed as $\mu L \cdot L^{-1}$.

At each harvest, all fruit that were deemed to be at the correct color development stage (> 50% red blush color) were picked and weighed. All fruit harvested on the different harvest dates were added together to determine total yield per tree. At each harvest, fruit drop was counted. Fruit sampling at harvest, as well as quality and maturity indexing were identical for all trials. A sample of 75 fruit per plot was randomly taken at each harvest and immediately brought to the laboratory at Stellenbosch University for evaluations. Samples were not taken at the third harvest for ‘Fuji’ and ‘Cripps’ Pink’ in the 2018/2019 season. All fruit were individually weighed. Fruit color was evaluated by scoring the percentage blush coverage of fruit, the blush color intensity scored according to the Pink Lady™ color chart for ‘Cripps’ Pink’ and the UNIFRUCO ‘Fuji’ color chart for ‘Fuji’ apples. A subsample of 25 fruit from the first two harvests was packed and placed in regular atmosphere (RA) storage at -0.5 ºC for 12 weeks in commercial M4 cartons with a plastic liner. Another subsample of 25 fruit of the first two harvests was packed and treated with 1-MCP at the ExperiCo (Agri-Research Solutions) laboratory (Stellenbosch, Western Cape, South Africa) within 24 hours of harvest. After 1-MCP treatment, fruit were placed in RA storage at -0.5 ºC for 12 weeks in commercial M4 cartons with a plastic liner. The remaining 25 fruit were used to evaluate fruit quality parameters and maturity indexing at harvest. Fruit ground color was scored according to the UNIFRUCO color chart for apples and pears (Paper 1). Greasiness was scored from 0 to 3, where 0 indicates no greasiness, 1 slight greasiness, 2 moderate greasiness and 3 severe greasiness. Fruit firmness was measured using a GÜSS texture analyzer (Güss electronic model GS 20, Strand, South Africa) fitted with an 11.1 mm tip, and starch breakdown determined using the UNIFRUCO starch conversion chart (Paper 1). Total soluble solids (TSS) content was determined using a digital refractometer (Model PR 32-α, Atago Co., Ltd., Tokyo, Japan).

After 12 weeks of RA storage, both 1-MCP treated and untreated fruit ground color and greasiness were evaluated. Fruit samples were then left at room temperature (± 20 ºC) for seven days to simulate shelf-life, where after fruit were re-evaluated for ground color, greasiness, firmness, starch breakdown and TSS as described for fruit at harvest.

Statistical analysis. All data were analyzed using SAS Enterprise guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA) using a 5 x 2 split-plot design, where five rates of ACC applied in the orchard was the main effect, followed by a post-harvest 1-MCP treatment or untreated control as the sub-treatment. A Least Significant Difference (LSD) test was performed when treatment effects in the ANOVA were significant ($P<0.05$).

Results

'Cripps' Pink' 2017/2018 season: Following the 400 $\mu L \cdot L^{-1}$ ACC application, IEC of fruit increased from 16 hours and continued to increase until the last sampling time 40 hours after application (Table 6). The IEC of untreated control fruit was consistently low irrespective of the sampling time. The IEC of ACC treated fruit was similar to the untreated control fruit only one hour before and one hour after treatment application (Table 6).

When the percentage fruit qualifying as Pink Lady™ was averaged over the three harvests and weighted according to harvest proportion, a significant increase in Pink Lady™ apples was seen compared to the control with the three higher ACC rates, whereas the control and 100 $\mu L \cdot L^{-1}$ ACC were statistically similar (Table 7). The combined percentage fruit classed as 'Cripps' Pink' from untreated control trees did not differ significantly from any of the ACC treatments, however, 100 $\mu L \cdot L^{-1}$ ACC had significantly more 'Cripps' Pink' classed fruit than the three higher ACC dosages. A general decrease in the combined percentage 3rd class fruit was observed with increasing ACC rate. In this case, the control and 100 $\mu L \cdot L^{-1}$ ACC were statistically similar, 200 $\mu L \cdot L^{-1}$ ACC did not differ from 100 $\mu L \cdot L^{-1}$ ACC or from the two higher rates, which also did not differ significantly from each other (Table 7). There were no significant differences found between treatments for percentage blush coverage at the first harvest (Table 8). At the second harvest, all fruit treated with ACC had significantly lower blush coverage than the control, except for 300 $\mu L \cdot L^{-1}$ ACC, which did not differ significantly from the control nor the other treatments (Table 9). At the third harvest, there was also no significant difference in blush percentage between treatments (Table 10). The percentage of fruit considered suitable to be marketed as Pink Lady™ as well as the percentage of 'Cripps' Pink' and third-class apples did not differ significantly among treatments at any of the three harvest dates (Table 8, 9 and 10). At first and second harvest, most fruit were considered Pink Lady™, followed by 'Cripps' Pink' graded fruit and then third-class apples. At the third harvest, however, there was a more even distribution of fruit percentages among grading classes. The red color intensity of fruit did not differ significantly from the control irrespective of treatment or harvest date (Table 8, 9 and 10). A decrease in red color intensity was observed from the first harvest to the last harvest date, a trend which was also visible for percentage blush coverage of fruit. When the blush percentage was averaged over the entire crop and weighted according to harvest proportion, an increase in total percentage blush coverage was observed with increasing ACC rate (Table 11).

Yield per tree was reduced by the 400 $\mu L \cdot L^{-1}$ ACC, although not significantly so compared to the 300 $\mu L \cdot L^{-1}$ ACC (Table 11). All ACC treatments advanced harvest time except 100 $\mu L \cdot L^{-1}$ compared to the control, as seen in the percentage of the total yield harvested at the first harvest date (Table 11). This trend was reversed at the third harvest date whereas at the second harvest date similar percentages were picked. An increase in ACC rate caused significantly more fruit to drop before the first harvest, especially at the highest rate of ACC at 400 $\mu L \cdot L^{-1}$ (Table 12). The additional fruit that dropped did not differ at the second harvest, but before the third harvest fewer fruit had dropped from ACC treated trees than from the control except for the 100 $\mu L \cdot L^{-1}$ ACC treatment.

At the first harvest, the higher rates of ACC resulted in significantly yellower ground color compared to the untreated control and fruit treated with 100 $\mu L \cdot L^{-1}$ (Table 13). Slight greasiness was observed at the first harvest date on fruit treated with 200, 300 and 400 $\mu L \cdot L^{-1}$ ACC, increasing with increasing rate, but 200 $\mu L \cdot L^{-1}$ ACC did not differ from the control. Starch conversion was increased by all ACC treatments compared to the untreated control, with 200, 300 and 400 $\mu L \cdot L^{-1}$ more so than 100 $\mu L \cdot L^{-1}$ (Table 13). TSS however did not differ between treatments at first harvest (Table 13) nor did the average fruit weight (Table 14). Fruit firmness was reduced by ACC treatments, but not significantly so for the 100 $\mu L \cdot L^{-1}$ ACC application (Table 14). At the second harvest date, no differences were found in ground color or TSS of fruit (Table 15). However, fruit with slight greasiness increased as ACC rate increased, although it did not differ significantly from the control when 100 or 200 $\mu L \cdot L^{-1}$ ACC were applied. Fruit starch conversion was increased by all ACC applications (Table 15). Fruit firmness was not significantly affected in fruit from the second harvest (Table 14). Fruit weight decreased with increasing ACC concentration, except for 200 $\mu L \cdot L^{-1}$ ACC, which was statistically similar to the control (Table 14). The highest rate of ACC (400 $\mu L \cdot L^{-1}$) resulted in the lowest fruit weight but was not significantly different from the 100 and 300 μL·L^{-1} ACC treatments.

After 12 weeks RA storage at -0.5 ºC, the ground color of 1-MCP treated fruit from the first harvest were greener for the two highest ACC rates, while there was no difference in ground color between 1-MCP treated and untreated fruit for the two lower ACC rates and the control fruit (Table 16). Fruit from the second harvest were yellower following the three higher ACC rates and 1-MCP treatment (Table 17). Immediately after cold storage, no significant differences were observed in the incidence of slight greasiness in fruit from the first harvest, while fruit from the second harvest had 1.8% fruit with slight greasiness following the 1-MCP treatment compared to the non-treated fruit with 0.8% (Table 17).

After 12 weeks RA storage at -0.5 ºC and seven days shelf-life at 20 ºC, the ground color of 1-MCP treated fruit was greener on both harvest dates (Table 18). Increasing rates of ACC resulted in yellower ground color. The percentage first harvest fruit with slight greasiness after storage and shelf-life tended to be lower after 1-MCP treatment except for the 100 μL·L^{-1} ACC treated and control fruit (Table 19). Fruit from the second harvest that had been treated with ACC had very low levels of slight greasiness when treated with post-harvest 1-MCP compared to fruit not treated with 1-MCP with a higher percentage slight greasiness. Control fruit, however, did not differ significantly with 1-MCP treatment in slight greasiness percentage (Table 19). Starch breakdown at the end of seven days shelf-life increased significantly with increased ACC rate and was lower in fruit treated post-harvest with 1-MCP (Table 20). All fruit from the second harvest displayed starch breakdown of 100% after shelf-life (data not shown). A general decrease in first harvest fruit firmness with increasing rates of ACC was noted after shelf-life, while treatment with 1-MCP significantly maintained fruit firmness compared to the untreated control (Table 20). TSS did not differ between ACC treated fruit from the first harvest, but it was higher in 1-MCP treated fruit. This was also the case in the second harvest, while TSS was lower in ACC treated fruit than in control fruit. (Table 20 and 21).

'Cripps' Pink' 2018/2019 season: Fruit IEC increased significantly with increasing rate of ACC application as well as over time (Table 22). Although IEC started increasing rapidly 72 hours after application it was still not significantly higher than before application of ACC. The highest rate of ACC (400 μL·L^{-1}) more than doubled the IEC compared to the control

'Cripps' Pink' fruit. IEC of fruit samples taken from 200 μL·L^{-1} ACC was greater than that of control fruit and less than 400 μL·L^{-1} ACC treated fruit, although not statistically so.

When the percentage fruit qualifying as Pink Lady™ was averaged over the first two harvest dates and weighted according to harvest proportion, a significant increase in Pink Lady™ apples was seen compared to the control with ACC application (Table 23). ACC applied at the highest rate differed significantly from the two lower rates, but not 300 μL·L^{-1} ACC, which differed significantly from 100 μL·L^{-1} ACC, but not 200 μL·L^{-1} ACC. The two lower ACC rates were statistically similar in IEC. There were, however, no significant differences found in the combined percentage fruit classed as 'Cripps' Pink' nor third class fruit, irrespective of treatment (Table 23). ACC significantly increased percentage blush compared to the control at first harvest, regardless of the rate applied (Table 24). There was also an increase in percentage fruit classified as Pink Lady™ as ACC rate increased. The highest rate of ACC (400 μL·L^{-1}) had the greatest percentage Pink Lady™ fruit at 80% compared to the control of 56%. A general decrease in 'Cripps' Pink' fruit was observed as ACC rate increased at first harvest, except for 100 μL·L^{-1} ACC, which was 10% greater than that of the control samples. Although percentage 'Cripps' Pink' apples from 200 μL·L^{-1} and 300 μL·L^{-1} ACC were lower than control samples, it was not statistically different from the control or 400 μL·L^{-1} ACC, the latter being significantly lower than the control (Table 24). The control fruit sample contained the highest percentage third-class fruit at first harvest. All treatments of ACC significantly decreased the percentage third-class fruit at first harvest, except 100 μL·L^{-1} ACC, which was lower but statistically similar to the control. No significant differences were observed in red color intensity at first harvest (Table 24). At second harvest, there was no significant difference among treatments for percentage blush, percentage Pink Lady™ fruit, percentage 'Cripps' Pink' apples, percentage third-class fruit, or red color intensity (Table 25). When blush percentage was averaged over the first two harvests and weighted according to harvest proportion, an increasing trend in total percentage blush coverage was observed with increasing ACC rate (Table 26).

The harvest was advanced by the two higher rates of ACC compared to the control and two lower rates that had a similar percentage fruit picked at the first harvest (Table 26). The second harvest was similar for all treatments, but the third harvest was smaller for the two higher ACC rates, while the 100 μL·L^{-1} ACC did not differ from the control, and 100 and 200 μL·L^{-1} ACC did not differ from each other. The total yield per tree did not differ between treatments (Table 26). The number of fruit that dropped before first commercial harvest was only increased by the 400 $\mu L \cdot L^{-1}$ ACC compared to the untreated control (Table 27).

Ground color was more yellow at the first harvest after all the ACC treatments except the lowest rate of ACC, with an increase in yellowing with increasing rate of ACC (Table 27). No greasiness was observed on 'Cripps' Pink' fruit at first harvest (data not shown) and TSS was also not affected. Starch breakdown at the first harvest significantly increased with increased rates of ACC except for the 100 $\mu L \cdot L^{-1}$ ACC treatment (Table 27). Also, at the second harvest, ACC induced a yellower ground color except in 100 $\mu L \cdot L^{-1}$ ACC treated fruit (Table 28). Greasiness was absent in fruit at the second harvest, but TSS was increased by all ACC treatments. Starch conversion was advanced by all ACC treatments except 100 $\mu L \cdot L^{-1}$ ACC, and the starch conversion was linearly advanced with an increasing rate of ACC (Table 28). Average fruit weight was not affected at either harvest date (Table 29). Although no significant differences were found in fruit firmness at the first harvest, firmness at the second harvest was reduced by 400 $\mu L \cdot L^{-1}$ ACC (Table 29).

After RA storage at -0.5 ºC for 12 weeks, fruit from both harvest dates had a more yellow ground color with increased ACC rate from ACC 200 $\mu L \cdot L^{-1}$ (Table 30). First harvest fruit treated with 1-MCP had a greener ground color than that of untreated fruit, whereas no significant difference was found in this regard for second harvest fruit after cold storage. No greasiness was observed after storage in fruit from either harvest (data not shown).

After 12 weeks of RA storage and seven days simulated shelf-life, the three highest ACC concentrations showed increased yellow ground color compared to the control and lowest ACC rate (Table 30). However, 1-MCP treatment significantly retarded yellowing of ground color compared to untreated fruit from both harvest dates. An interaction between ACC and 1-MCP treatment was seen for greasiness incidence of both harvest dates after cold storage and seven days shelf-life (Table 31). In both cases greasiness was very low and only occurred in fruit not treated with 1-MCP and the higher ACC rates. Fruit starch breakdown was at 100% after cold storage and shelf-life for fruit for both harvests (data not shown). ACC did not influence fruit firmness of first harvest fruit after shelf-life, but 1-MCP retained fruit firmness compared to untreated fruit (Table 32). TSS varied slightly between ACC treatments, but not between 1-MCP or no 1-MCP treatments. Second harvest fruit treated with ACC displayed decreased firmness from 200 $\mu L \cdot L^{-1}$ when comparing to the untreated control, except for 300

$\mu L \cdot L^{-1}$ ACC. Again, fruit treated with 1-MCP were firmer than untreated fruit after shelf-life. TSS decreased with increasing ACC rate in the second harvest (Table 32).

'Fuji' 2018/2019 season: At first harvest, there was no significant difference between the percentage blush coverage nor the red color intensity of fruit regardless of treatment (Table 33). 'Fuji' apples treated with 200 $\mu L \cdot L^{-1}$ ACC had a significantly lower percentage fruit with 50% blush coverage or more compared to the control and the other ACC treatments, while all other ACC treatments were statistically similar to the control. At second harvest, there were significant differences among all ACC treatments for the percentage blush coverage, the percentage of fruit with more than 50% blush as well as red color intensity of fruit (Table 34). None of the treatments for any of the before mentioned parameters differed significantly from the control. Although the highest rate of ACC (400 $\mu L \cdot L^{-1}$) had the greatest percentage blush (62%), the highest percentage of fruit with 50% blush or more (91%) and the most intense red color (3.2) of all treatments including the control (not significantly different), no definite trend was visible among other treatments.

When the blush percentage was averaged over the first two harvests and weighted according to harvest proportion, no significant differences were found in total percentage blush coverage irrespective of treatment (Table 35). There was, however, an increase in the combined percentage fruit with more than 50% blush with increasing ACC rate. The highest rate of ACC had significantly more fruit with 50% or more blush than any other treatment. The three lower rates of ACC did not differ significantly from each other. The untreated control had significantly fewer fruit with 50% or more blush than all other treatments, except for 200 $\mu L \cdot L^{-1}$ ACC that did not differ significantly from the control (Table 35). All ACC applications advanced fruit harvesting except the 200 $\mu L \cdot L^{-1}$ ACC application. ACC at 300 and 400 $\mu L \cdot L^{-1}$ resulted in an increase in the number of fruit that dropped to the ground before harvest with the number being doubled by the 400 $\mu L \cdot L^{-1}$ compared to the 300 $\mu L \cdot L^{-1}$ treatment, ACC at 400 $\mu L \cdot L^{-1}$ thus reducing yield compared to the untreated control and ACC at 200 $\mu L \cdot L^{-1}$ (Table 35 and 36).

The ground color of the fruit picked during the first harvest was not affected by ACC treatment (Table 36), whereas at the second harvest, ground color of control fruit and ACC 400 $\mu L \cdot L^{-1}$ were similar but less yellow than other treatments (Table 37). No greasiness of the fruit peel was observed at either harvest date (data not shown). The fruit starch breakdown was significantly higher in ACC treated fruit except for the 100 $\mu L \cdot L^{-1}$ ACC, especially at the first harvest where the highest ACC concentration (400 $\mu L \cdot L^{-1}$) displayed starch breakdown almost double that of the untreated control (Table 36). At the second harvest, only fruit from the highest ACC rates (300 $\mu L \cdot L^{-1}$ and 400 $\mu L \cdot L^{-1}$) had greater starch breakdown than the untreated control (Table 37). No differences were found in TSS at either harvest date (Table 36 and 37). Fruit weight was not affected by the ACC treatment at both harvest dates (Table 38). At both harvest dates, fruit firmness declined as ACC concentration increased, although at the first harvest, differences were not statistically significant and only 400 $\mu L \cdot L^{-1}$ ACC significantly reduced fruit firmness at the second harvest compared to the control (Table 38).

After 12 weeks RA cold storage, background color of fruit sampled at both harvest dates was more yellow with increasing ACC concentration except for 200 $\mu L \cdot L^{-1}$ ACC and 100 $\mu L \cdot L^{-1}$ ACC at second harvest, whereas 1-MCP significantly reduced yellowing compared to the untreated fruit (Table 39). No greasiness incidence was observed in 'Fuji' apples from either harvest date directly after cold storage (data not shown).

After RA cold storage and seven days shelf-life, ground color and greasiness were re-evaluated on the same fruit. There were no significant differences in the ground color of ACC treated fruit and the untreated control for the first harvest, however 1-MCP treatment of the same fruit displayed a less yellow ground color compared to untreated fruit (Table 40). A significant interaction between ACC and 1-MCP was observed in ground color of second harvest fruit (Table 41). Generally, ACC advanced yellowing with increasing concentration, whereas 1-MCP effectively reduced yellowing, but fruit from the highest ACC rate (400 $\mu L \cdot L^{-1}$) displayed a background color similar to the untreated control. None of the 1-MCP treated fruit differed significantly from the control. Fruit from both harvest dates, had very little greasiness after shelf-life and it did not differ between ACC treatments (Table 40). However, treatment with 1-MCP completely prevented greasiness incidence of the peel after shelf-life. Starch conversion was 100% in fruit from both harvest dates after seven days simulated shelf-life (data not shown). Although there was no significant difference between the firmness of ACC treated fruit and the control, 1-MCP treatment significantly maintained fruit firmness of fruit from both harvest dates (Table 42). No significant differences were found in TSS in fruit from either harvest.

Discussion

Fruit ripening and anthocyanin biosynthesis in apple are ethylene-mediated processes (Saure, 1990) and ethylene synthesis is autocatalytic (Ireland et al., 2014). The increase in IEC following ACC application and subsequent advanced maturity of the fruit during both seasons, demonstrates that ACC was successfully absorbed by treated fruit and converted to ethylene. Although IECs increased with increasing ACC rate, results show that fruit remained preclimacteric until seven days after application, indicated by an IEC of less than 1 $\mu l \cdot L^{-1}$ (Doerflinger et al., 2019). Whale et al. (2008) reported that 2-chloroethylphosphonic acid (Ethephon) application at 280 $g \cdot ha^{-1}$ five weeks before harvest on 'Cripps' Pink' apples over two consecutive seasons resulted in elevated levels of IEC as well as respiration rate of fruit. Interestingly, various authors have also reported increases in ACC oxidase (ACO) and ACC synthase (ACS) activities with preharvest Ethephon application (Larrigaudiere et al., 1996; Li et al., 2002; Zhu et al., 2015). According to Larrigaudiere et al. (1996), Ethephon applied to 'Starking Delicious' apples two weeks before harvest not only increased red color and internal ethylene of fruit until 30 days after harvest, but also caused a sharp rise in ACO activity before harvest, followed by a rapid decline shortly thereafter. Li et al. (2002) reported similar findings on 'Fuji' apples. According to Zhu et al. (2015), Ethephon stimulated both ACO and ACS activity in banana fruit, whereas 1-MCP suppressed their activity.

'Cripps' Pink'. The average blush percentage, when averaged over the total crop and weighted according to harvest proportion, showed a clear increase in total percentage blush coverage with increasing ACC rate for both seasons. This was also reflected in the percentage of fruit classified as Pink Lady™ over all harvest dates. The similarity of blush coverage among treatments within a harvest is not surprising, since fruit were harvested according to a minimum of 40% blush coverage and thus samples taken from picking bags are expected to have similar red blush percentages irrespective of treatment. In both seasons, a general decline in percentage blush was observed as harvest progressed. This is especially evident in the first season, where percentage blush ranged from 50% to 52% at first harvest, 39% to 46% at the second harvest and 30% to 36% at the third harvest of 'Cripps' Pink' apples. A similar tendency was reflected in the percentage fruit classified as Pink Lady™ during the 2018 season. Although there were no significant differences observed in the percentage fruit graded as Pink Lady™ in the first season, an increase in the percentage Pink Lady™ classified fruit with increasing rate of ACC was seen at first harvest of the second season. The difference between the two seasons in this regard is due to fruit from the 2018 season having an overall blush coverage between 39% and

52% for the first two harvest dates and thus almost all fruit that were picked qualified to be marketed as Pink Lady™ apples. This implies that any difference due to ACC treatment would be small and insignificant. In the second season, however, percentage blush coverage ranged from 32% to 46% for the two harvest dates and thus, generally fewer fruit were graded as Pink Lady™. Therefore, a more distinct treatment effect was seen, where the percentage Pink Lady™ fruit increased as ACC rate increased. Whale et al. (2008) applied Ethephon; 280 $g \cdot ha^{-1}$, an ethylene-releasing compound, as well as AVG (124.5 $g \cdot ha^{-1}$), an amino acid inhibiting ethylene synthesis, alone and in combination to ‘Cripps’ Pink’ apples 5 weeks before harvest. Samples were taken randomly from fruit that were strip picked at harvest and fruit color and other quality parameters were analyzed. During the first season, the blush percentage of fruit treated with Ethephon alone was 30% more (78%) than that of control fruit (48%). Anthocyanin concentration also increased significantly compared to the control and the percentage of fruit meeting Pink Lady™ export standards increased by 36%. Similar results were obtained in the second season of the same study. According to Whale and Singh (2007), two peaks of color development occur naturally during apple fruit growth, both coinciding with an increase in endogenous ethylene in the fruit. The first and minor peak occurs around seven days after full bloom (DAFB) followed by a second, economically important peak after 168 DAFB in ‘Cripps’ Pink’ apples. In their study on ‘Cripps’ Pink’ apples in Australia, fruit were strip harvested during both seasons, but in the second season sampling continued weekly until four weeks after harvest. During the second season, an increase from 16% to 44% blush coverage was noted from the start of the second peak to commercial harvest at 187 DAFB, although color development continued until 215 DAFB. However, according to Steyn et al. (2009), red color in ‘Cripps’ Pink’, increased every time there was a cold front and decreased again between cold fronts, with the ability to develop color gradually increasing. Stern et al. (2010) studied ‘Cripps’ Pink’ apples in Israel, and the blush percentage of fruit was increased by circa 20% with the use of synthetic auxin 2,4-DP, applied at 50 $mg \cdot L^{-1}$ at either 45 or 75 DAFB. The increase in blush color as well as increased blush intensity in the same study was a direct result of increased anthocyanin content due to increased ethylene production (Stern et al., 2010).

The importance of the correct timing of harvest is reflected in the linear trend observed in yield distribution in both seasons, where the percentage fruit picked during the first harvest was more than double for the highest rate of ACC than for the untreated control. The inverse was true for the percentage of total yield picked at the final harvest date. Since ‘Cripps’ Pink’ apples are harvested with a minimum of 40% blush, one can infer from the shift in yield distribution that ACC treatment effectively increased color development with increasing concentration, an observation supported by the increased combined percentage blush coverage over harvest. The same trend in yield distribution was observed in Paper 1, where ACC (200 $\mu L \cdot L^{-1}$) was applied to 'Cripps' Pink' apples one and two weeks before harvest. In the same paper, when aminoethoxyvinylglycine (AVG) (125 $mg \cdot L^{-1}$) was applied to 'Cripps' Pink' apples at one and two weeks before harvest, the inverse was observed in terms of yield distribution. This is due to AVG inhibiting, while ACC enhances, ethylene synthesis. Therefore, the clear indication of a shift in harvest time to an earlier window is attributed to ACC application causing elevated ethylene levels and subsequently faster ripening and increased color development of fruit. Yield was decreased to some extent in the first season, where the highest rate of ACC led to more than four times the number of fruit that dropped before the first harvest compared to the untreated control, and explains the decrease in total yield with increasing ACC concentration in the 2018 season. In earlier, unpublished research, it was noted that the less colored inner canopy fruit were more mature and tended to drop when treated with ACC (Reynolds, personal communication). The first harvest was done when the orchard was released for harvest and the ACC treated trees, especially at the higher rates, should have been harvested earlier than the orchard release date. Since the ACC treated fruit were considerably more mature at harvest, it would be interesting to know what the color would have looked like if fruit were picked at 30% starch breakdown, as in the case of the control rather than 70%. One can infer that ethylene did not per se necessarily increase anthocyanin synthesis and thus red color development, but rather advanced fruit maturity with red color development possibly being a coincidental correlated phenomenon.

Advanced fruit maturity is also reflected in parameters such as the fruit ground color, fruit firmness, greasiness, and starch breakdown. Ground color of ACC treated fruit was consistently yellower at harvest and after 12 weeks RA storage at -0.5 ºC and seven days shelf-life during both seasons compared to the untreated control. Although the differences in ground color were relatively small, postharvest 1-MCP treatment prevented yellowing of fruit during both seasons. Significantly higher firmness of postharvest 1-MCP treated fruit from both seasons after 12 weeks RA storage and seven days shelf-life further confirms the efficacy of 1-MCP in delaying fruit maturation. 1-MCP has approximately ten times the affinity for ethylene receptors than ethylene itself (Tassoni et al., 2006), and therefore prevents the ripening process by binding to ethylene receptor sites, thereby preventing ethylene's action (Iqbal et al., 2017).

Falagán and Terry (2020) also noted no change in fruit firmness of 1-MCP treated 'Aroma' apples after 1 and 1.5 months in cold storage, compared to a 10% loss in firmness in control fruit. The same observation was noted by Crouch et al. (2005) on 'Cripps' Pink' apples treated with 1-MCP postharvest and stored in RA at -0.5 °C for four months followed by seven days shelf-life at 15 °C. Flesh firmness of treated apples was maintained and the authors saw a difference of more than 1 kg firmness between treated and control fruit. Both Whale et al. (2008) and Stern et al. (2010), on the other hand, found that although application of 2-chloroethylphosphonic acid (Ethephon) and 2,4-DP, respectively, to 'Cripps' Pink' apples caused an increase in ethylene synthesis in the fruit, fruit firmness remained unaffected at harvest.

In our trials, greasiness was especially problematic in the second harvest of the first season, with the highest rate of ACC (400 $\mu L \cdot L^{-1}$) leading to 74% of fruit with slight greasiness compared to a mere 12% for the untreated control. Although the high incidence of greasiness in the first season could indicate advanced fruit maturity at harvest, it is more likely a scoring error since greasiness usually develops and worsens after storage. Greasiness at all other evaluation times was not problematic. Postharvest treatment with 1-MCP greatly reduced greasiness incidence after 12 weeks storage and seven days shelf-life of both harvest dates and both seasons. Significant increases of starch breakdown in both seasons with increasing ACC concentration starting from 200 $\mu l \cdot L^{-1}$ ACC also indicates advanced maturity at harvest. This further supports the notion of an earlier shift in harvest date. Stern et al. (2010) similarly found that application of the synthetic auxin 2,4-DP, which enhances fruit maturity by upregulating ethylene synthesis, caused an increase in starch breakdown of treated fruit at harvest.

Fuji. Red blush coverage of ACC treatments did not differ significantly from the control at first or second harvest, and all treatments and controls displayed blush coverage over 50%, except 200 $\mu l \cdot L^{-1}$ ACC at first harvest (49%). The same 200 $\mu l \cdot L^{-1}$ ACC treatment was also the only treatment to differ significantly from the control regarding the percentage of fruit with 50% blush or more (62% compared to the control of 77%). The percentage of fruit with 50% or greater blush coverage of all other treatments and controls ranged between 73% and 91%. Unlike in 'Cripps' Pink', the similarity in percentage blush coverage at harvest across treatments is probably not due to the method of sampling, as the combined percentage blush coverage over the first two harvests did not differ significantly between treatments. Some 'Fuji' clones have a much different pigmentation pattern than the two peaks described earlier in this section. In these clones, color is often at a peak quite a while before harvest and then decreases towards harvest. Therefore, with these clones, it is questionable whether ACC would improve color. It is, however, uncertain whether Kiku belongs to this group of strains. However, when combining the percentage fruit with 50% or more blush coverage over both harvests and weighting it according to harvest proportion, an increase in fruit with 50% blush or more is observed with increasing ACC rate. The highest ACC rate resulted in 28.3% more fruit with 50% blush or more than the control. Li et al. (2002) found that applying 100 $mg \cdot L^{-1}$ 2-chloroethylphosphonic acid (Ethephon) to 'Fuji' apples four weeks before harvest significantly increased anthocyanin accumulation and red color development of the apples. Although not statistically different from the control, a general small decrease in percentage of fruit with 50% blush or more was observed in the fruit sampled at first harvest with increasing rates of ACC. The same trend was, however, not observed at the second harvest, therefore this small decrease is probably due to sampling variation.

A definite shift in harvest maturity was reflected in the larger percentage of ACC treated fruit picked at the first harvest compared to the control. This is especially true for the two higher rates of ACC (300 $\mu l \cdot L^{-1}$ and 400 $\mu l \cdot L^{-1}$), where 45% and 43% of fruit were picked at first harvest, respectively, compared to 28% for the untreated control. One can infer that a third harvest would have been necessary for control trees, since more than two-thirds of fruit were picked at the second harvest. There was a slight decrease in total yield with ACC treatment, except for 200 $\mu l \cdot L^{-1}$ ACC. The decrease in yield of 26.5 kg per tree for 400 $\mu l \cdot L^{-1}$ ACC treated trees compared to control trees is probably due to the extensive fruit drop induced by ACC. Fruit drop may indicate advanced fruit maturity at harvest and these trees should have been harvested earlier.

Higher levels of starch breakdown at harvest with increasing ACC indicates that ACC treated fruit were physiologically more mature at harvest. The highest rate of ACC (400 $\mu L \cdot L^{-1}$) displayed starch breakdown almost double that of the untreated control at first harvest and one and a half times at second harvest. Using 1-MCP retained fruit maturity and storability. This is clearly shown by fruit treated with 1-MCP having slightly greener ground color than untreated fruit. Whale and Singh (2007) attributes the yellowing of apple ground color to increased activity of chlorophyllase and chlorophyll degradation peroxidase due to higher ethylene concentrations, causing chlorophyll degradation. Since 1-MCP prevents the action of ethylene by binding to its receptors (Tomala et al., 2020), enzyme activity is subsequently lowered, and fruit ground color is maintained. Fruit treated with 1-MCP were also consistently and significantly firmer than fruit not treated with 1-MCP after storage and shelf-life.

Larrigaudiere et al. (1996) similarly found that Ethephon application to 'Starking Delicious' apples consistently decreased firmness over two seasons, both at harvest and 60 days after harvest. Yellower ground color and decreased firmness are both indications of advanced fruit maturity.

'Fuji' apples were less responsive to ACC than 'Cripps' Pink' apples. This could be since 'Fuji' apples produce lower levels of ethylene (Doerflinger et al., 2019) and could therefore respond less strongly to changes in IEC due to upregulation of ethylene biosynthesis via ACC application. However, data collection over more than one season would be beneficial to confirm the consistency of this observation.

Conclusion

Application of ACC two weeks before harvest resulted in a shift in yield distribution, where a larger portion of the total harvest was picked at the first harvest with increasing ACC concentration, compared to the untreated control. The rapid spike in IEC in the few days following ACC application, as well as the plateau in IEC from 7 to 21 days after application demonstrated in 2019, may indicate ACC application closer, around one week, to harvest as a possible alternative to application two weeks before harvest, however this was not found in Paper 1. Application closer to harvest will allow producers to make a well-informed decision on whether ACC application for color development is necessary. Our data indicate an increase in blush color for the whole harvest with ACC treatment of 'Cripps' Pink' apples, but not for 'Fuji' apples. The earlier shift in harvest with increasing ACC concentration indicates that a greater proportion of fruit on the tree had blush coverage of over 40% when compared to untreated control trees, although fruit maturity was severely affected and fruit should have been harvested earlier. In future studies, the sampling method should be adjusted to give a more holistic portrayal of color distribution on each tree at commercial harvest. This could be achieved by strip picking trees (or large scaffold branches) at optimum harvest and scoring the blush coverage of all harvested apples, instead of randomly selecting sample fruit from picking bags at each harvest. Furthermore, fruit should also be harvested at the optimum fruit maturity (30% starch breakdown) for each respective treatment. Loss of fruit firmness, yellower ground color and increased starch breakdown indicate that ACC advanced fruit maturity at harvest and after storage of both cultivars for both seasons, especially at the higher rates. This could potentially decrease long-term storability of fruit, again emphasizing that it is critical to harvest fruit at the correct maturity. These trials indicate that postharvest treatment of fruit with 1-MCP counteracted the negative ripening effects of preharvest ACC application. Long-term storage will, however, not be feasible if fruit were picked over mature. It seems as though ACC had a more pronounced effect on ‘Cripps’ Pink’ compared to that of ‘Fuji’ apples. It would therefore be beneficial to further evaluate the combination of preharvest ACC application and postharvest 1-MCP treatment on other bi-color apple cultivars of different ethylene sensitivities over multiple seasons to determine to what extent the effect of ACC is cultivar specific. ACC applied at 200 $\mu l \cdot L^{-1}$, followed by postharvest 1-MCP treatment showed potential for improving color development of bi-color apple cultivars. It would be worthwhile to investigate alternative approaches to improving color development without advancing fruit maturity, such as defoliation or reflective mulches.

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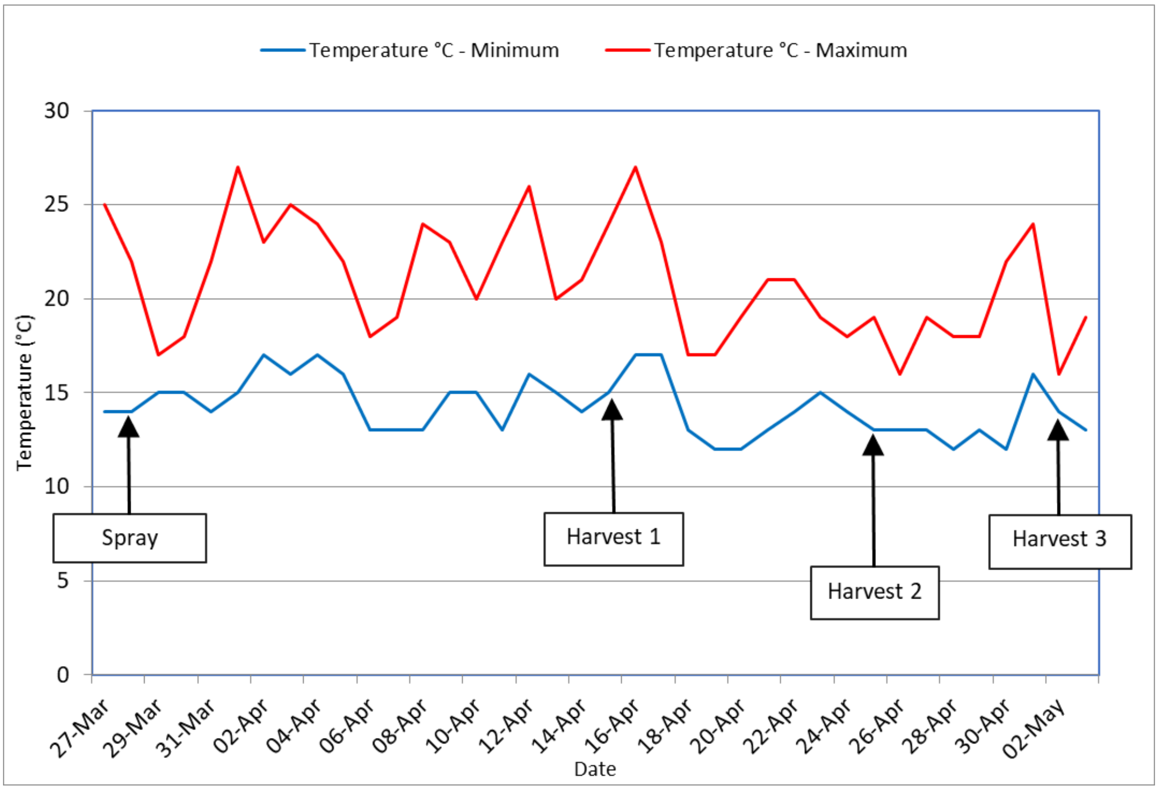

Fig. 2. Weather data at the 'Cripps' Prink' trial site in 2018 at Applegarth, Grabouw, South Africa

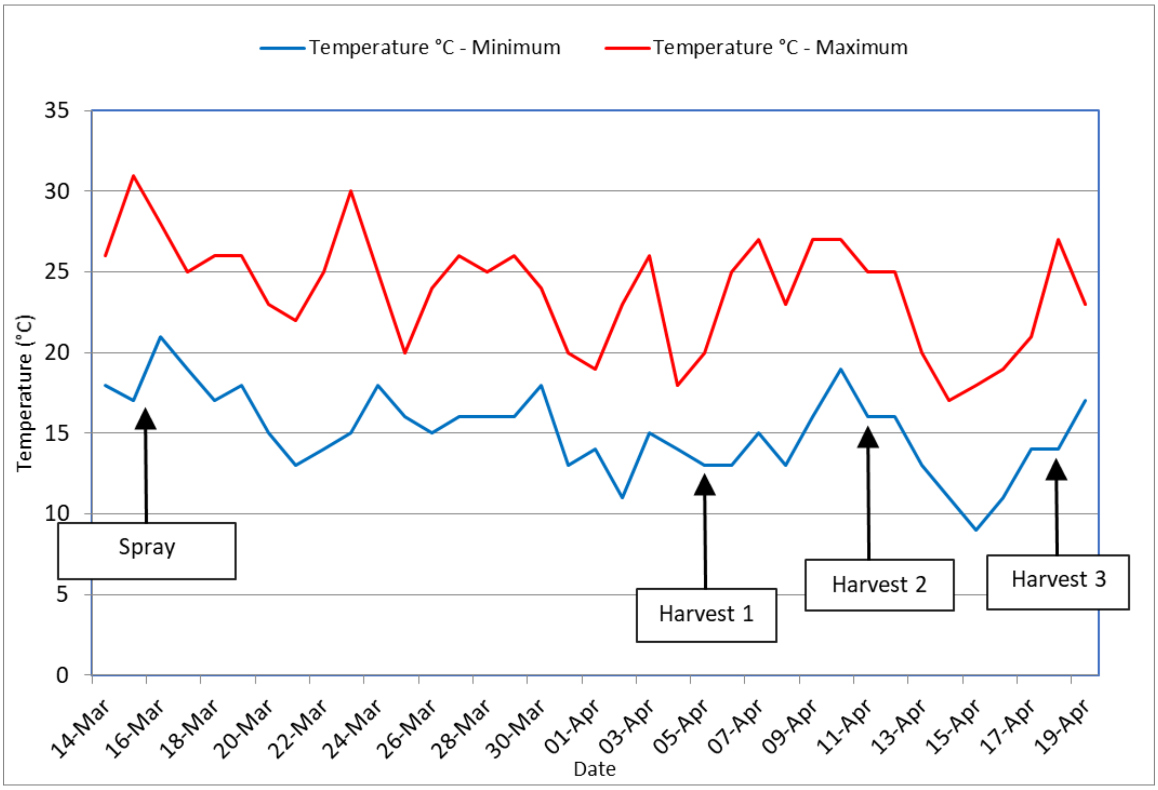

Fig. 3. Weather data at the 'Cripps' Pink' trial site in 2019 at Lourensford, Somerset West, South Africa

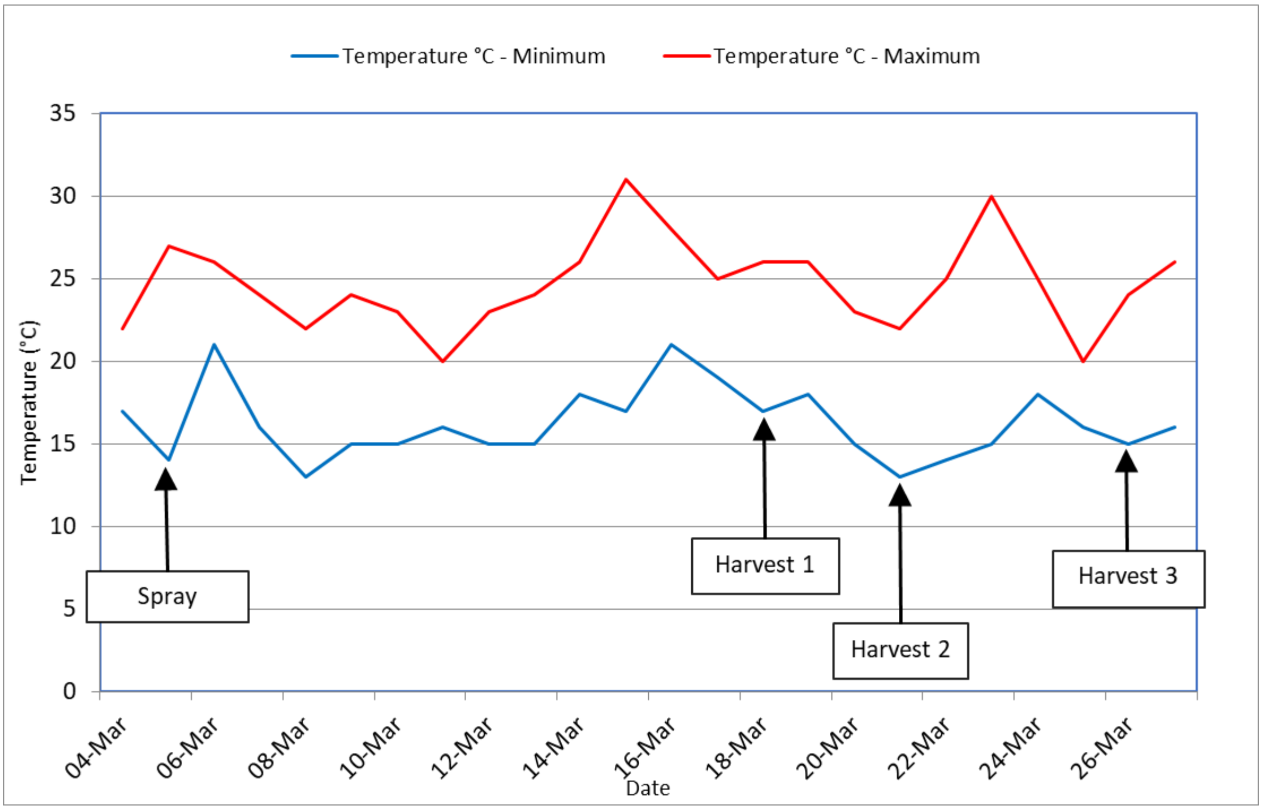

Fig. 4. Weather data at the 'Fuji'' trial site in 2019 at Graymead, Elgin, South Africa

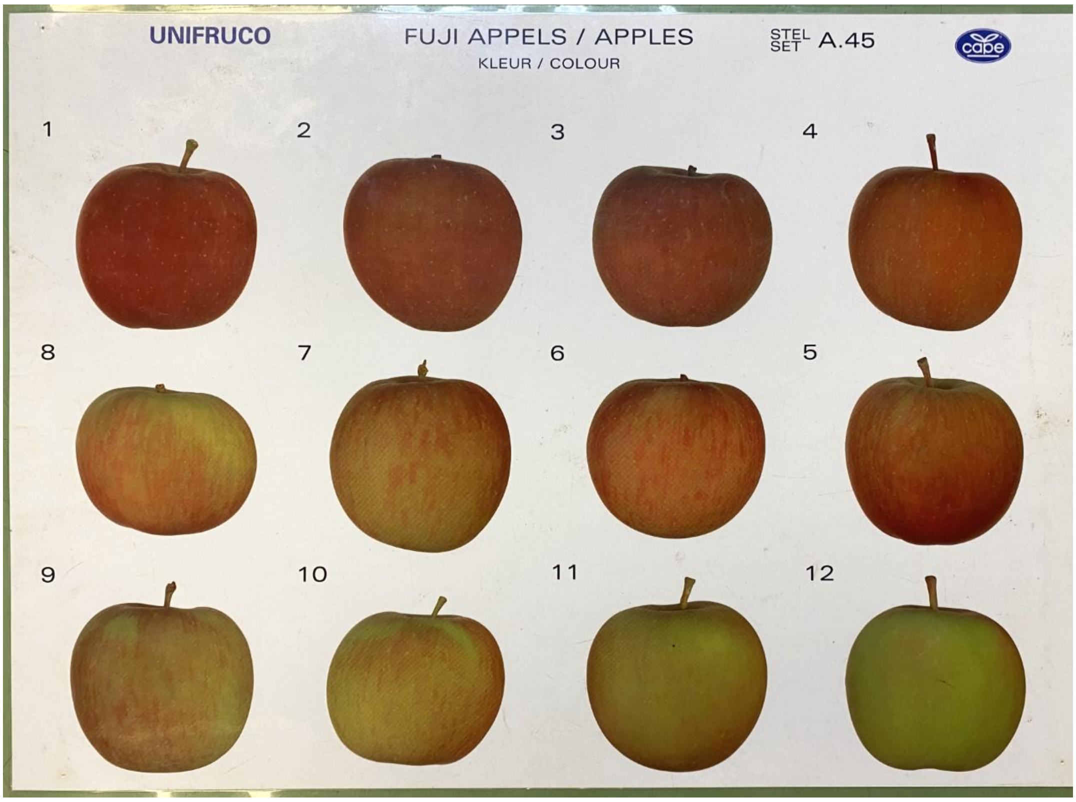

Fig. 4. UNIFRUCO color chart used to determine blush color intensity of ‘Fuji’ apples.

Table 1. Treatment summary for trials done with 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-methylcyclopropene (1-MCP) on ‘Cripps’ Pink’ (2018 and 2019 seasons) and ‘Fuji’ apples (2019 season) on Applegarth, Elgin, Lourensford, Somerset West, and Graymead, Vyeboom, respectively.

Orchard treatment	Post-harvest treatment
Untreated control	1-MCP or No 1-MCP
ACC* (100 $\mu L \cdot L^{-1}$) at 2 wbh**	1-MCP or No 1-MCP
ACC* (200 $\mu L \cdot L^{-1}$) at 2 wbh	1-MCP or No 1-MCP
ACC* (300 $\mu L \cdot L^{-1}$) at 2 wbh	1-MCP or No 1-MCP
ACC* (400 $\mu L \cdot L^{-1}$) at 2 wbh	1-MCP or No 1-MCP

*ACC applied with surfactant: Villa 51 @ 6 $\mu L \cdot L^{-1}$

**Weeks before harvest (wbh)

Table 2. Summary of the dates of 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-methylcyclopropene (1-MCP) treatment applications and harvests for ‘Cripps’ Pink’ in the 2018 season on Applegarth, Elgin.

Activity	Date
Application of ACC	28 March 2018
Harvest 1	16 April 2018
Application of 1-MCP	17 April 2018
Harvest 2	25 April 2018
Application of 1-MCP	26 April 2018
Harvest 3	2 May 2018

Table 3. Summary of the dates of 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-methylcyclopropene (1-MCP) treatment applications and harvests for ‘Cripps’ Pink’ and ‘Fuji’ apples in the 2019 season on Lourensford, Somerset West, and Graymead, Vyeboom, respectively.

Activity	Date	
	‘Cripps’ Pink’	‘Fuji’
Application of ACC	15 March 2019	5 March 2019
Harvest 1	5 April 2019	18 March 2019
Application of 1-MCP	8 April 2019	19 March 2019
Harvest 2	11 April 2019	21 March 2019
Application of 1-MCP	12 April 2019	22 March 2019
Harvest 3	18 April 2019	26 March 2019

Table 4. Summary of the dates of internal ethylene concentration (IEC) sampling of control and 1-aminocyclopropane-1-carboxylic acid (ACC) 400 $\mu L \cdot L^{-1}$ for 'Cripps' Pink' apples in the 2018 season at Applegarth, Elgin.

Time of sampling:	Date
Pre-foliar application	28 March 2018
1 hour post application	28 March 2018
16 hours post application	29 March 2018
24 hours post application	29 March 2018
40 hours post application	30 March 2018

Table 5. Summary of the dates of internal ethylene concentration (IEC) sampling of control, 1-aminocyclopropane-1-carboxylic acid (ACC) 200 $\mu L \cdot L^{-1}$ and ACC 400 $\mu L \cdot L^{-1}$ for 'Cripps' Pink' apples in the 2019 season at Lourensford, Somerset West.

Time of sampling	Date
Pre-foliar application	15 March 2019
24 hours post application	16 March 2019
48 hours post application	17 March 2019
72 hours post application	18 March 2019
120 hours post application	20 March 2019
168 hours post application	22 March 2019
First harvest post application	5 April 2019

Table 6. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) (400 $\mu L \cdot L^{-}1$) on internal ethylene concentration (IEC) of 'Cripps' Pink' apples over time at Applegarth, Elgin (2018).

Treatment	Time after application (h)	IEC ($\mu L \cdot L^{-1}$)	
Control	-1	0.0	c
Control	1	0.1	c
Control	16	0.1	c
Control	24	0.0	c
Control	40	0.1	c
ACC 400	-1	0.0	c
ACC 400	1	0.0	c
ACC 400	16	0.3	b
ACC 400	24	0.3	b
ACC 400	40	0.7	a
Treatment		***< 0.0001***	
Time after application		***< 0.0001***	
*Treatment*Time after application*		***< 0.0001***	

Table 7. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on the color distribution combined over three harvests for 'Cripps' Pink' apples at Applegarth, Elgin (2018).

Treatment	Combined percentage Pink Lady™* over harvest	Combined percentage 'Cripps' Pink'* over harvest	Combined percentage 3rd class* fruit over harvest
Control	58.4 b	24.5 ab	17.1 a
ACC 100	59.7 b	26.5 a	13.8 ab
ACC 200	70.4 a	20.0 b	9.6 bc
ACC 300	73.2 a	19.7 b	7.1 c
ACC 400	74.5 a	19.8 b	5.7 c
Significance level	***0.0010***	***0.0314***	***0.0043***
LSD 5%	*9.0*	*5.3*	*6.3*

*Blush >40% = Pink Lady™; blush 20-40% = 'Cripps' Pink'; blush <20% = 3rd class

Table 8. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on red color development of 'Cripps' Pink' apples at first harvest at Applegarth Elgin (2018).

Treatment	Percentage blush coverage	Percentage Pink Lady™*	Percentage 'Cripps' Pink'*	Percentage 3rd class fruit*	Red color intensity**
Control	52.3 ns	90.1 ns	8.6 ns	1.4 ns	7.2 ns
ACC 100	51.4	86.6	12.0	1.4	7.0
ACC 200	51.7	89.0	9.2	1.9	7.5
ACC 300	51.8	87.4	12.0	0.5	7.0
ACC 400	50.4	82.8	13.7	3.5	7.3
Significance level	*0.9330*	*0.4339*	*0.5454*	*0.1594*	*0.4098*
LSD 5%	-	-	-	-	-

*Blush >40% = Pink Lady™; blush 20-40% = 'Cripps' Pink'; blush <20% = 3rd class

**Scored from 1 to 12 with 1 no color and 12 intense color (Paper 1)

Table 9. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on red color development of ‘Cripps’ Pink; apples at second harvest at Applegarth, Elgin (2018).

Treatment	Percentage blush coverage	Percentage Pink Lady™*	Percentage ‘Cripps’ Pink’*	Percentage 3rd class fruit*	Red color intensity**
Control	46.4 a	73.7 ns	22.1 ns	4.3 ns	6.0 ns
ACC 100	39.3 b	61.6	30.9	7.5	5.3
ACC 200	41.7 b	67.5	26.3	6.3	5.4
ACC 300	43.1 ab	72.4	23.2	4.3	5.6
ACC 400	42.2 b	68.4	26.6	5.0	5.4
Significance level	***0.0219***	*0.1037*	*0.1429*	*0.4258*	*0.1649*
LSD 5%	*4.1*	-	-	-	-

*Blush >40% = Pink Lady™; blush 20-40% = ‘Cripps’ Pink’; blush <20% = 3rd class

**Scored from 1 to 12 with 1 no color and 12 intense color (Paper 1)

Table 10. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on red color development of ‘Cripps’ Pink’ apples at third harvest at Applegarth Elgin (2018).

Treatment	Percentage blush coverage	Percentage Pink Lady™*	Percentage ‘Cripps’ Pink’*	Percentage 3rd class fruit*	Red color intensity**
Control	29.5 ns	38.3 ns	33.8 ns	27.8 ns	3.8 ns
ACC 100	29.3	36.7	35.8	27.5	3.7
ACC 200	34.9	50.9	27.5	21.6	4.4
ACC 300	35.6	48.2	32.2	19.7	4.5
ACC 400	31.5	42.3	36.5	21.2	4.0
Significance level	*0.0841*	*0.0954*	*0.1973*	*0.2953*	*0.0943*
LSD 5%	-	-	-	-	-

*Blush >40% = Pink Lady™; blush 20-40% = ‘Cripps’ Pink’; blush <20% = 3rd class

**Scored from 1 to 12 with 1 no color and 12 intense color (Paper 1)

Table 11. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 µL·L^{-1}) on the combined percentage blush coverage over harvest, yield distribution and total yield of 'Cripps' Pink' apples at Applegarth, Elgin (2018).

Treatment	Combined percentage blush coverage over harvest		Total yield per tree (kg)		Percentage harvest 1		Percentage harvest 2		Percentage harvest 3	
Control	38.6	c	160.1	a	23.0	c	29.9	ns	47.1	a
ACC 100	39.2	bc	170.7	a	29.3	c	38.6		32.1	b
ACC 200	43.3	ab	156.6	a	39.9	b	35.0		25.2	b
ACC 300	45.0	a	147.4	ab	47.9	b	31.2		20.9	b
ACC 400	46.4	a	128.0	b	64.0	a	28.4		7.6	c
Significance level	***0.0022***		***0.0353***		***<.0001***		*0.0967*		***<.0001***	
LSD 5%	*4.3*		*27.1*		*8.9*		-		*12.4*	

Table 12. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 µL·L^{-1}) on fruit drop of 'Cripps' Pink' apples at Applegarth, Elgin (2018).

Treatment	Number of fruit picked up below tree harvest 1		Number of fruit picked up below tree harvest 2		Number of fruit picked up below tree harvest 3	
Control	26.6	c	16.7	ns	12.2	a
ACC 100	47.4	c	23.5		9.3	ab
ACC 200	54.2	c	24.3		6.8	bc
ACC 300	89.0	b	22.8		4.8	c
ACC 400	122.1	a	21.9		4.9	c
Significance level	***<.0001***		*0.2213*		***0.0002***	
LSD 5%	*27.6*		-		*3.3*	

Table 13. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 µL·L^{-1}) on fruit maturity of 'Cripps' Pink' apples at first harvest at Applegarth Elgin (2018).

Treatment	Ground color*		Percentage fruit with slight greasiness		Average fruit TSS (%)		Average fruit percentage starch conversion	
Control	3.1	c	0	c	13.0	ns	37.3	c
ACC 100	3.2	c	0	c	13.1		48.3	b
ACC 200	3.5	ab	8	bc	13.1		66.3	a
ACC 300	3.4	b	11.6	ab	13.3		65.8	a
ACC 400	3.6	a	19.2	a	13.3		74.1	a
Significance level	***<.0001***		***0.0008***		*0.4454*		***<.0001***	
LSD 5%	*0.1*		*9.5*		-		*10.7*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 14. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on fruit weight and firmness of 'Cripps' Pink' apples at first harvest at Applegarth Elgin (2018).

Treatment	Average fruit weight (g)				Average fruit firmness (kg)			
	First harvest		Second harvest		First harvest		Second harvest	
Control	144.3	ns	149.5	a	8.61	a	8.21	ns
ACC 100	142.7		143.6	bc	8.54	ab	8.23	
ACC 200	142.8		144.6	ab	8.35	b	8.13	
ACC 300	145.7		141.7	bc	8.38	b	8.08	
ACC 400	141.5		138.5	c	8.37	b	8.00	
Significance level	*0.6495*		***0.0081***		***0.047***		*0.1582*	
LSD 5%	-		***5.7***		*0.21*		-	

Table 15. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on fruit maturity of 'Cripps' Pink' apples at second harvest at Applegarth Elgin (2018).

Treatment	Ground color*		Percentage fruit with slight greasiness		Average fruit TSS (%)		Average fruit percentage starch conversion	
Control	3.3	ns	12.0	c	12.9	ns	44.8	b
ACC 100	3.2		24.8	bc	13.3		67.8	a
ACC 200	3.3		32.4	bc	12.9		69.9	a
ACC 300	3.3		63.2	a	13.0		74.6	a
ACC 400	3.3		74.3	a	13.0		73.2	a
Significance level	*0.293*		***<.0001***		*0.357*		***<.0001***	
LSD 5%	-		*15.4*		-		*8.9*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 16. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on the ground color of 'Cripps' Pink' apples at first fruit post 12 weeks cold storage (2018).

Treatment	1-MCP	Ground color*	
Control	No	3.23	f
Control	Yes	3.35	cdef
ACC 100	No	3.24	ef
ACC 100	Yes	3.28	ef
ACC 200	No	3.52	bc
ACC 200	Yes	3.43	cde
ACC 300	No	3.64	ab
ACC 300	Yes	3.33	def
ACC 400	No	3.77	a
ACC 400	Yes	3.49	bcd
ACC		*<.0001*	
MCP		*0.0144*	
*ACC*MCP*		***0.0039***	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 17. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on fruit greasiness of 'Cripps' Pink' apples at first and second harvest and ground color of second harvest fruit post 12 weeks cold storage (2018).

Treatment	Ground color*		Percentage fruit with slight greasiness			
			First harvest		Second harvest	
ACC						
Control	3.04	b	0.6	ns	1.0	ns
ACC 100	3.04	b	1.2		0.6	
ACC 200	3.33	a	0.8		1.2	
ACC 300	3.35	a	2.4		1.4	
ACC 400	3.32	a	2.0		0.8	
MCP						
No	3.16	b	1.1	ns	0.2	b
Yes	3.27	a	1.8		1.8	a
ACC	***<.0001***		*0.4371*		*0.8268*	
MCP	***0.0020***		*0.3198*		***0.0008***	
*ACC*MCP*	*0.1003*		*0.8349*		*0.5962*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 18. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on fruit ground color of 'Cripps' Pink' apples at first and second harvest post 12 weeks cold storage and 7 days shelf life (2018).

Treatment	Ground color*			
	First harvest		Second harvest	
ACC:				
Control	3.72	c	3.59	c
ACC 100	3.83	bc	3.62	c
ACC 200	3.89	b	3.84	b
ACC 300	3.94	ab	3.88	ab
ACC 400	4.05	a	3.96	a
MCP:				
No	4.00	a	3.90	a
Yes	3.77	b	3.66	b
ACC	***<.0001***		***<.0001***	
MCP	***<.0001***		***<.0001***	
*ACC*MCP*	*0.4273*		*0.5156*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 19. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on fruit greasiness of 'Cripps' Pink' apples at first and second harvest post 12 weeks cold storage and 7 days shelf life (2018).

Treatment	1-MCP	Percentage fruit with slight greasiness			
		First harvest		Second harvest	
Control	No	2.2	d	4.5	bc
Control	Yes	2.0	d	1.2	c
ACC 100	No	2.0	d	11.0	b
ACC 100	Yes	2.0	d	1.2	c
ACC 200	No	9.0	bc	22.3	a
ACC 200	Yes	3.2	d	0.0	c
ACC 300	No	12.5	ab	25.5	a
ACC 300	Yes	1.6	d	0.4	c
ACC 400	No	13.3	a	21.6	a
ACC 400	Yes	5.2	cd	0.8	c
ACC		*<.0001*		*0.0014*	
MCP		*<.0001*		*<.0001*	
*ACC*MCP*		***0.0045***		***0.0005***	

Table 20. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 µL·L^{-1}) and 1-methylcyclopropene (1-MCP) on starch breakdown, TSS and fruit firmness of 'Cripps' Pink' first harvest apples post 12 weeks cold storage and 7 days shelf life (2018).

Treatment	Average fruit percentage starch conversion		Average fruit TSS (%)		Fruit firmness (kg)	
ACC:						
Control	91.1	c	13.54	ns	7.50	a
ACC 100	95.4	b	13.54		7.44	ab
ACC 200	98.8	a	13.39		7.39	ab
ACC 300	100.0	a	13.26		7.22	c
ACC 400	100.0	a	13.31		7.29	bc
MCP:						
No	97.9	a	13.30	b	6.38	b
Yes	96.3	b	13.51	a	8.36	a
ACC	***<.0001***		*0.0505*		***0.0023***	
MCP	***0.0082***		***0.0035***		***<.0001***	
*ACC*MCP*	*0.1661*		0.6019		*0.1405*	

Table 21. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 µL·L^{-1}) and 1-methylcyclopropene (1-MCP) on fruit TSS and fruit firmness of 'Cripps' Pink second harvest apples post 12 weeks cold storage and 7 days shelf life (2018).

Treatment	Average fruit TSS (%)		Fruit firmness (kg)	
ACC:				
Control	13.52	a	7.47	a
ACC 100	13.18	b	7.40	a
ACC 200	13.05	b	7.24	b
ACC 300	13.24	b	7.13	bc
ACC 400	13.15	b	7.08	c
MCP:				
No	13.06	b	6.36	b
Yes	13.39	a	8.17	a
ACC	***0.0002***		***<.0001***	
MCP	***<.0001***		***<.0001***	
*ACC*MCP*	*0.2423*		*0.9512*	

Table 22. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (200 $\mu L \cdot L^{-1}$ and 400 $\mu L \cdot L^{-1}$) on internal ethylene concentration (IEC) of 'Cripps' Pink' apples over time at Lourensford, Somerset West (2019).

Treatment	IEC ($\mu L \cdot L^{-1}$)
ACC:	
Control	0.3 b
ACC 200	0.5 ab
ACC 400	0.7 a
Time after ACC application (h):	
0	0.0 c
24	0.0 c
48	0.1 c
72	0.4 bc
120	0.7 ab
168	1.1 a
504	1.1 a
Treatment	***0.0183***
Time after application	***<.0001***
*Treatment*Time after application*	*0.2952*

Table 23. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on the color distribution combined over the first two harvests for 'Cripps' Pink' apples at Lourensford, Somerset West (2019).

Treatment	Combined percentage Pink Lady™* over two harvests	Combined percentage 'Cripps' Pink'* over two harvests	Combined percentage 3rd class* fruit over two harvests
Control	26.8 d	17.2 ns	9.0 ns
ACC 100	39.5 c	25.2	7.9
ACC 200	47.4 bc	23.9	3.5
ACC 300	52.7 ab	22.5	3.9
ACC 400	65.1 a	22.9	4.4
Significance level	***<.0001***	*0.2391*	*0.0899*
LSD 5%	*12.6*	-	-

*Blush >40% = Pink Lady™; blush 20-40% = 'Cripps' Pink'; blush <20% = 3rd class

Table 24. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on red color development of 'Cripps' Pink' apples at first harvest at Lourensford, Somerset West (2019).

Treatment	Percentage blush coverage	Percentage Pink Lady™*	Percentage 'Cripps' Pink'*	Percentage 3rd class fruit*	Red color intensity**
Control	36.3 b	56.2 c	27.1 b	16.8 a	4.6
ACC 100	43.4 a	65.6 bc	37.1 a	13.7 a	5.3
ACC 200	44.5 a	72.9 ab	23.9 bc	3.2 b	5.6
ACC 300	45.7 a	78.5 ab	17.4 bc	4.1 b	5.8
ACC 400	46.3 a	79.8 a	16.2 c	4.0 b	5.9
Significance level	***0.0376***	***0.0086***	***0.0007***	***0.0012***	*0.0723*
LSD 5%	*6.8*	*14.1*	*9.8*	*7.7*	-

*Blush >40% = Pink Lady™; blush 20-40% = 'Cripps' Pink'; blush <20% = 3rd class

**Scored from 1 to 12 with 1 no color and 12 intense color (Paper 1)

Table 25. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on red color development of 'Cripps' Pink' apples at second harvest at Lourensford, Somerset West (2019).

Treatment	Percentage blush coverage	Percentage Pink Lady™*	Percentage 'Cripps' Pink'*	Percentage 3rd class fruit*	Red color intensity**
Control	31.9 ns	42.8 ns	37.3 ns	19.9 ns	4.1 ns
ACC 100	35.8	53.3	38.1	8.6	4.2
ACC 200	36.3	51.3	41.5	7.1	4.5
ACC 300	34.8	44.2	47.8	8.0	4.1
ACC 400	36.2	50.2	43.6	6.2	4.1
Significance level	*0.6372*	*0.5876*	*0.2753*	*0.1246*	*0.8411*
LSD 5%	-	-	-	-	-

*Blush >40% = Pink Lady™; blush 20-40% = 'Cripps' Pink'; blush <20% = 3rd class

**Scored from 1 to 12 with 1 no color and 12 intense color (Paper 1)

Table 26. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on yield distribution and fruit drop at first harvest of ‘Cripps’ Pink’ apples at Lourensford, Somerset West (2019).

Treatment	Combined percentage blush coverage over first two harvests		Percentage harvest 1		Percentage harvest 2		Percentage harvest 3		Total yield per tree (kg)	
Control	15.7	c	15.92	b	32.29	ns	51.80	a	76.65	ns
ACC 100	20.6	bc	17.46	b	34.78		47.76	ab	75.98	
ACC 200	24.2	b	23.64	b	36.19		40.17	b	91.61	
ACC 300	30.6	a	38.80	a	34.29		26.91	c	81.79	
ACC 400	31.2	a	38.49	a	36.02		25.49	c	69.17	
Significance level	***<.0001***		***<.0001***		*0.9081*		***<.0001***		***0.0781***	
LSD 5%	***5.4***		*9.81*		-		*10.64*		-	

Table 27. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on fruit drop and maturity of ‘Cripps’ Pink’ apples at first harvest at Lourensford, Somerset West (2019).

Treatment	Number of fruit picked up below tree		Ground color*		Average fruit TSS (%)		Average fruit percentage starch conversion	
Control	17.5	bc	2.71	c	12.73	ns	36.9	c
ACC 100	13.5	c	2.87	bc	12.90		42.4	bc
ACC 200	14.5	c	3.01	ab	12.75		50.1	b
ACC 300	22.5	ab	3.17	a	13.08		60.0	a
ACC 400	29.0	a	3.24	a	12.93		64.1	a
Significance level	***0.0001***		***0.0026***		*0.6436*		***<.0001***	
LSD 5%	*6.6*		*0.28*		-		*8.6*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 28. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on fruit maturity of 'Cripps' Pink' apples at second harvest at Lourensford, Somerset West (2019)

Treatment	Ground color*		Percentage fruit with no greasiness		Average fruit TSS (%)		Average fruit percentage starch conversion	
Control	2.79	c	100.0	ns	12.40	b	37.09	c
ACC 100	2.90	bc	100.0		13.23	a	37.20	c
ACC 200	3.22	ab	96.0		13.32	a	49.14	b
ACC 300	3.18	ab	100.0		13.26	a	54.62	ab
ACC 400	3.27	a	100.0		13.39	a	60.48	a
Significance level	***0.0132***		*0.4203*		***0.0002***		***<.0001***	
LSD 5%	*0.32*		-		*0.43*		*8.5*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 29. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on fruit weight and firmness of 'Cripps' Pink' apples at harvest at Lourensford, Somerset West (2019).

Treatment	Average fruit weight (g)				Average fruit firmness (kg)			
	First harvest		Second harvest		First harvest		Second harvest	
Control	106.6	ns	108.1	ns	9.30	ns	9.06	a
ACC 100	103.2		107.5		9.30		9.07	a
ACC 200	106.4		108.5		9.37		9.03	a
ACC 300	105.7		104.7		9.07		8.86	ab
ACC 400	104.2		104.8		9.02		8.63	b
Significance level	*0.8147*		*0.3722*		*0.0689*		***0.0102***	
LSD 5%	-		-		-		*0.27*	

Table 30. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on ground color of 'Cripps' Pink' apples at first and second harvest post 12 weeks cold storage and post shelf-life (2019).

Treatment	Ground color*							
	Post RA storage				Post shelf-life			
	First harvest		Second harvest		First harvest		Second harvest	
ACC								
Control	2.54	b	2.73	b	3.12	c	3.15	b
ACC 100	2.72	b	2.71	b	3.28	bc	3.20	b
ACC 200	2.98	a	3.14	a	3.44	ab	3.56	a
ACC 300	3.10	a	3.25	a	3.59	a	3.60	a
ACC 400	3.18	a	3.32	a	3.58	a	3.74	a
MCP								
No	2.98	a	3.03	ns	3.57	a	3.69	a
Yes	2.82	b	3.03		3.23	b	3.21	b
ACC	***<.0001***		***<.0001***		***0.0001***		***<.0001***	
MCP	***0.0371***		*0.9523*		***<.0001***		***<.0001***	
*ACC*MCP*	*0.4931*		*0.3906*		*0.7620*		*0.8230*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 31. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on fruit greasiness of 'Cripps' Pink' apples at first and second harvest post 12 weeks cold storage and 7 days shelf life (2019).

Treatment	1-MCP	Percentage fruit with no greasiness			
		First harvest		Second harvest	
Control	No	0.4	b	0.0	c
Control	Yes	0.0	b	0.0	c
ACC 100	No	0.0	b	0.0	c
ACC 100	Yes	0.0	b	0.0	c
ACC 200	No	0.4	b	2.4	bc
ACC 200	Yes	0.0	b	0.0	c
ACC 300	No	6.9	a	7.2	ab
ACC 300	Yes	0.0	b	0.0	c
ACC 400	No	9.8	a	9.3	a
ACC 400	Yes	0.0	b	0.0	c
ACC		*0.0002*		*0.0002*	
MCP		*<.0001*		*<.0001*	
*ACC*MCP*		***0.0002***		***0.0002***	

Table 32. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on fruit firmness and total soluble solids (TSS) of 'Cripps' Pink' apples post 12 weeks cold storage and 7 days shelf life (2019).

Treatment	Average fruit firmness (kg)		Average fruit TSS (%)	
	First harvest	Second harvest	First harvest	Second harvest
ACC:				
Control	8.41 ns	7.87 a	14.15 a	13.94 a
ACC 100	8.16	7.84 a	13.79 b	13.80 ab
ACC 200	8.13	7.61 b	14.15 a	13.70 abc
ACC 300	8.25	7.75 ab	13.89 ab	13.59 bc
ACC 400	8.13	7.59 b	13.61 b	13.43 c
MCP:				
No	7.41 b	6.75 b	13.88 ns	13.60 ns
Yes	9.02 a	8.71 a	13.95	13.78
ACC	*0.1587*	***0.0158***	***0.0076***	***0.0489***
MCP	***<.0001***	***<.0001***	*0.5433*	*0.1165*
*ACC*MCP*	*0.6980*	*0.3615*	*0.2239*	*0.9023*

Table 33. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on red color development of 'Fuji' apples at first harvest at Graymead Elgin (2019).

Treatment	Percentage blush coverage	Percentage greater than 50% blush	Red color intensity*
Control	53.9 ns	76.8 a	4.1 ns
ACC 100	53.8	80.1 a	4.1
ACC 200	49.4	61.9 b	4.7
ACC 300	51.5	73.4 a	4.4
ACC 400	51.3	72.8 a	4.6
Significance level	*0.0656*	***0.0216***	*0.0502*
LSD 5%	-	*10.9*	-

*Scored from 2 to 12 with 12 no color and 2 intense color (Fig. 4)

Table 34. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on red color development of 'Fuji' apples at second harvest at Graymead Elgin (2019).

Treatment	Percentage blush coverage		Percentage greater than 50% blush		Red color intensity*	
Control	59.2	ab	86.4	ab	3.5	abc
ACC 100	56.3	b	84.0	b	3.9	a
ACC 200	56.8	b	81.3	b	3.7	ab
ACC 300	59.8	ab	85.7	ab	3.4	bc
ACC 400	61.8	a	91.3	a	3.2	c
Significance level	***0.0256***		***0.0444***		***0.0135***	
LSD 5%	*3.6*		*6.4*		*0.4*	

*Scored from 2 to 12 with 12 no color and 2 intense color (Fig. 4)

Table 35. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on combined percentage blush coverage and percentage fruit with 50% or more blush coverage over two harvests, yield distribution and total yield per tree of 'Fuji' apples at Graymead, Grabouw (2019)

Treatment	Combined percentage blush coverage over first two harvests		Combined percentage fruit with > 50% blush coverage over first two harvests		Percentage harvest 1		Percentage harvest 2		Total yield per tree (kg)	
Control	57.9	ns	43.9	c	28.40	c	71.60	a	115.77	ab
ACC 100	55.8		55.7	b	40.87	ab	59.13	bc	108.22	bc
ACC 200	54.7		52.1	bc	33.88	bc	66.12	ab	131.99	a
ACC 300	56.2		60.3	b	45.16	a	54.84	c	108.71	bc
ACC 400	57.7		72.2	a	43.43	a	56.57	c	89.30	c
Significance level	*0.0695*		***0.0003***		***0.0003***		***0.0003***		***0.0069***	
LSD 5%	-		*11.5*		*7.67*		*7.67*		*21.57*	

Table 36. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on fruit fall and fruit maturity of 'Fuji' apples at first harvest at Graymead Elgin (2019).

Treatment	Number of fruit picked up below tree		Ground color*		Average fruit TSS (%)		Average fruit percentage starch conversion	
Control	18.9	c	2.0	ns	13.9	ns	30.0	d
ACC 100	16.5	c	2.2		14.2		36.0	cd
ACC 200	24.6	c	2.3		14.2		46.1	bc
ACC 300	100.0	b	2.2		14.0		54.8	ab
ACC 400	201.2	a	2.2		13.9		58.3	a
Significance level	***<.0001***		*0.0737*		*0.6400*		***<.0001***	
LSD 5%	*23.4*		-		-		*11.7*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 37. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on fruit maturity of 'Fuji' apples at second harvest at Graymead Elgin (2019).

Treatment	Ground color*		Average fruit TSS (%)		Average fruit percentage starch conversion	
Control	2.1	b	14.2	ns	32.2	b
ACC 100	2.6	a	14.6		34.9	b
ACC 200	2.5	a	14.6		34.3	b
ACC 300	2.5	a	14.2		48.8	a
ACC 400	2.0	b	14.4		46.7	a
Significance level	***<.0001***		*0.5550*		***0.0026***	
LSD 5%	*0.1*		-		*9.9*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 38. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) on fruit weight and firmness of 'Fuji' apples at first and second harvest at Graymead Elgin (2019).

Treatment	Average fruit weight (g)				Average fruit firmness (kg)			
	First harvest		Second harvest		First harvest		Second harvest	
Control	134.0	ns	122.0	ns	8.21	ns	9.06	a
ACC 100	133.9		128.7		8.32		9.07	a
ACC 200	129.4		120.6		8.11		9.03	a
ACC 300	130.6		122.9		8.04		8.86	ab
ACC 400	131.3		120.5		8.01		8.63	b
Significance level	*0.7310*		*0.1224*		*0.0682*		***0.0102***	
LSD 5%	-		-		-		*0.27*	

Table 39. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on the ground color of 'Fuji' apples for first and second harvest post 12 weeks cold storage (2019).

Treatment	Ground color*			
	First harvest		Second harvest	
ACC:				
Control	2.90	b	2.82	b
ACC 100	3.01	a	2.84	b
ACC 200	2.99	ab	2.87	b
ACC 300	3.05	a	3.03	a
ACC 400	3.04	a	3.03	a
MCP:				
No	3.05	a	2.96	a
Yes	2.95	b	2.88	b
ACC	***0.0136***		***<.0001***	
MCP	***0.0016***		***0.0107***	
*ACC*MCP*	*0.3045*		*0.1411*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 40. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on the ground color at first harvest and greasiness at first and second harvest of 'Fuji' apples post 12 weeks cold storage and 7 days shelf life (2019).

Treatment	Ground color*		Percentage fruit with no greasiness			
	First harvest		First harvest		Second harvest	
ACC:						
Control	3.2	ns	99.0	ns	99.8	ns
ACC 100	3.2		99.0		97.6	
ACC 200	3.1		98.6		99.8	
ACC 300	3.2		99.0		99.4	
ACC 400	3.2		99.6		97.8	
MCP:						
No	3.3	a	98.1	b	97.8	b
Yes	3.1	b	100.0	a	100.0	a
ACC	*0.5002*		*0.8017*		*0.2237*	
MCP	***<.0001***		***0.0002***		***0.0069***	
*ACC*MCP*	*0.3219*		*0.8017*		*0.2237*	

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 41. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on ground color development of 'Fuji' second harvest apples post 12 weeks cold storage at -0.5 °C and 7 days shelf life at 20 °C (2019).

Treatment	1-MCP	Ground color*
Control	No	3.1 c
Control	Yes	3.2 bc
ACC 100	No	3.3 ab
ACC 100	Yes	3.2 bc
ACC 200	No	3.1 c
ACC 200	Yes	3.1 c
ACC 300	No	3.4 ab
ACC 300	Yes	3.1 c
ACC 400	No	3.5 a
ACC 400	Yes	3.1 c
ACC		*0.0049*
MCP		*0.0017*
*ACC*MCP*		***0.0046***

*Scored from 0.5 to 5 with 0.5 being green and 5 indicating yellow (Paper 1)

Table 42. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration (100 – 400 $\mu L \cdot L^{-1}$) and 1-methylcyclopropene (1-MCP) on fruit firmness, starch breakdown and total soluble solids of 'Fuji' apples post 12 weeks cold storage at -0.5 °C and 7 days shelf life at 20 °C (2019).

Treatment	Average fruit firmness (kg)		Average fruit TSS (%)	
	First harvest	Second harvest	First harvest	Second harvest
<u>ACC:</u>				
Control	8.0 ns	7.9 ns	15.7 ns	15.7 ns
ACC 100	8.1	7.9	15.7	15.6
ACC 200	7.9	7.8	15.2	15.4
ACC 300	7.9	7.9	15.2	15.2
ACC 400	7.9	7.8	15.3	15.0
<u>MCP:</u>				
No	7.8 b	7.6 b	15.3 ns	15.2 ns
Yes	8.1 a	8.1 a	15.6	15.5
ACC	*0.1047*	*0.2752*	*0.0629*	*0.0915*
MCP	***<.0001***	***<.0001***	*0.0514*	*0.0901*
*ACC*MCP*	*0.5955*	*0.9759*	*0.9107*	*0.9071*

PAPER 3: The Use of Reflective Mulch to Improve Red Color Development in 'Fuji' and 'Rosy Glow' Apples

Additional index words. anthocyanin, radiation, Lumilys®, ColorIt

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Abstract.

One of the major challenges that apple growers in Mediterranean-type climates experience is poor and erratic red color development of red apple cultivars. An increase in net installation to decrease sunburn, wind and hail damage, decreases radiation reaching fruit and therefore also red color development. The purpose of this study was to evaluate the efficacy of two commercially available reflective mulches, viz. Lumilys® and ColorIt, installed four to five weeks before harvest to increase reflected photosynthetic active radiation (PAR) and subsequently red color development on two commercially important bi-color apple cultivars, viz. 'Fuji Brak' (Kiku) and 'Rosy Glow'. In addition, the effect of these mulches under shade netting was evaluated on both cultivars. Reflective mulches increased the maximum percentage of reflected incident light by 38.1 – 46.0% compared to the control grassed alleyway. Fruit red color coverage was enhanced by both mulches, in both cultivars, although it was more evident in 'Rosy Glow' than in 'Fuji' apples and fruit found in lower canopy positions than upper canopy positions, which showed red color development similar to or greater than that of control fruit from upper canopy positions. A similar trend was seen for red color intensity in 'Rosy Glow' apples. The yields of both 'Fuji' and 'Rosy Glow' apples were unaffected by mulch installation. Varying results of fruit quality and maturity indices, such as fruit size, starch breakdown, fruit firmness and ground color may indicate that the effect of increased light from reflective mulches on fruit quality and maturity may be cultivar specific and should be further investigated.

The success of bi-colored apples on the market relies heavily on the percentage of red blush color coverage of the fruit. Fruit with a greater blushed surface area of optimum red color intensity are preferred by consumers and return greater profits (Dar et al., 2019; Kays, 1999;

Tijskens et al., 2011). A lack of red color development is often the result of fruit not exposed to sufficient light, as anthocyanin biosynthesis is a light-dependent pathway (Saure, 1990). This is evident in fruit found at lower and inner tree canopy positions where light levels are low (Hamadziripi et al., 2014).

In apple fruit, the few weeks leading up to harvest are the most important for red color development. During this time, various environmental and developmental factors contribute to anthocyanin biosynthesis and chlorophyll degradation (Whale et al., 2008). The two most important environmental factors are light and temperature (Ubi, 2004). The importance of light stretches beyond its requirement to mediate the light-dependent anthocyanin synthetic pathway. Light is also necessary for optimal photosynthesis and carbohydrate production to sustain the high sink demand for fruit (Fujii and Kennedy, 1985). Low temperatures are directly correlated to anthocyanin biosynthesis, whereas high temperatures negate anthocyanin accumulation (Ubi, 2004). Optimum temperatures for red color development vary among apple cultivars. Lin-Wang et al. (2011) report that high air temperatures significantly reduced the expression of genes required for anthocyanin synthesis, but that transcription is increased by even a single night of low temperatures. They also reported that red color development in 'Cripps' Pink' occurred optimally between 16 and 25 °C. The combined result of favorable environmental and developmental factors is an increased anthocyanin:chlorophyll ratio, which gives the fruit its characteristic blushed appearance.

Various methods to increase red color development in apples have been evaluated. These include reflective mulching, application of plant growth regulators (PGRs), evaporative cooling as well as pruning and thinning of the tree canopy to improve light distribution. Although using the correct planting system and row direction, in combination with correct pruning strategies remains the first approach used to maximize light interception and distribution, if the right planting system and rootstock combination was not chosen at establishment of the orchard, pruning cannot rectify light distribution without compromising yield (Palmer et al., 1992). Evaporative cooling, although effective (Iglesias et al., 2002), is not a sustainable practice in South Africa, where water shortages are frequent and water quality would need to be carefully monitored (Van den Dool, 2006). Application of PGRs such as ethephon (Shafiq et al., 2014; Ubi, 2004), methyl jasmonate (Shafiq et al., 2011) and abscisic acid (Jiang and Joyce, 2003) have also shown potential, but hasten fruit ripening (Li et al., 2002; Stern et al., 2010), therefore decreasing storability of fruit and often also negatively affect fruit eating quality. This was also shown in Paper 2 where various rates of foliar 1-

aminocyclopropane-1-carboxylic acid (ACC) application to 'Cripps' Pink' and 'Fuji' apples were made two weeks before harvest. Although color development occurred earlier, the firmness of fruit from both cultivars decreased, starch breakdown was advanced, and a yellower ground color was observed compared to the respective untreated controls. Higher levels of ethylene were also measured from fruit treated with higher rates of ACC (Paper 2), all indicative of advanced fruit maturity.

Installing a reflective ground cover in the orchard alleyway has gained popularity in recent years and significantly increases the incidence of scattered photosynthetic active radiation (PAR) and ultra-violet (UV) light into the canopy, therefore increasing light interception by fruit and thus increasing anthocyanin biosynthesis (Leão de Sousa and Sánchez, 2020). Privé (2008) in Canada, installed Extenday™ from full bloom to three weeks after harvest to evaluate the changes in the canopy microclimate of 'Gala' apples. Extenday™ successfully increased reflected PAR, especially into the lower canopy, up to nine times compared to the control grassed alley. It was also noted that the reflective mulch increased the levels of reflected PAR in both the middle and top of the tree canopy, albeit to a much lower extent than the bottom canopy. Schuhknecht et al. (2018) reported significant increases in class one pack-out of 'Braeburn' apples due to better color development using Extenday™ and Lumilys® reflective mulches in Germany. Both mulches were installed seven weeks before harvest and resulted in 49% and 36% fruit with more than 25% surface red color for Extenday™ and Lumilys®, respectively, whereas the control grassed alley produced only 5% fruit with more than 25% surface color. In Portugal, Leão de Sousa and Sánchez (2020) similarly found that installing a UV stabilized polypropylene reflective material 3 weeks before harvest not only increased PAR reflection into the lower canopy but also increased fruit red color intensity of 'Fuji' apples and positively affected photosynthetic efficiency of the tree.

In addition, the increasing use of protective nets in South African orchards to reduce sunburn, wind or hail damage, decreases light interception, and therefore decreases red color development (Mupambi et al., 2018; Smit et al., 2007). Installing reflective mulch may hold great potential to counteract this effect.

This paper reports on the potential of two commercially available reflective ground cover mulches to increase red color development in 'Fuji Brak' (Kiku) apples in the Elgin region and 'Rosy Glow' apples in the Ceres region. We also report on the effect of the respective reflective mulches when used in combination with either draped or permanent shade net.

Materials and Methods

Plant material and site description. Trials were conducted during the 2018/2019 season in commercial 'Fuji' (Brak) and 'Rosy Glow' apple orchards to compare the efficacy of two reflective mulches to improve red color development. In addition, trials were included where trees were under permanent shade netting or draped net. The two trials on 'Fuji' were conducted at Graymead (34° 1' 32.1" S, 19˚ 7' 49.3" E; 331 m. a. s. l.) in the Elgin, Grabouw, Vyeboom, Villiersdorp (EGVV) region in the Western Cape, South Africa. Trees on M793 rootstock were planted 4 m x 2 m in 2006 in a north-south row direction and trained to a central leader system. One trial was conducted where 20% white draped net (Drape Net® SA (Pty) Ltd., Nashdale, NSW, Australia) was installed and the same trial repeated in a section of the orchard without draped net. The 'Rosy Glow' trials were conducted on the farm Paardekloof (33° 15' 40.0" S, 19° 15' 43.4" E; 1690 m. a. s. l.) in the Witzenberg Valley near Ceres in the Western Cape, South Africa. The rows are in a north-south direction and trees on MM109 rootstock with M9 interstem were planted at a spacing of 3.5 m x 1.25 m in 2011. The trees are trained to a central leader system. One trial was conducted in a section of the orchard that was covered by permanent, 20% white shade net (PlusNet/Geotex, Aureus, Randfontein, South Africa), while the same trial was repeated in a part of the orchard that was uncovered.

Treatments and experimental layout. The treatments and trial layout for all four trials were the same. The two white, UV stabilized, woven reflective fabrics were Lumilys® (Beaulieu International Group, Warrem, Belgium) and ColorIt (Proline, Hastings, Hawke's Bay, New Zealand) and were compared to an untreated, grassed alley row. A randomized complete block design was used with six replications. Mulch strips of 12 m long and 3.3 m and 3.0 m wide for Lumilys® and ColorIt, respectively, were installed four to five weeks before commercial harvest. Mulch strips spanned across approximately six trees and two trees were left open on either side as a buffer between treatments (Fig. 1). Mulch treatments were applied on both sides of a row. In the case of the untreated control, 12 m of grassed alleyway was used. Two uniform trees were selected in the center of the treatment plot on the east and west sides of the rows and were tagged for sampling at harvest, as shown in Fig. 2. The mulch for the

'Fuji' trial was installed on 19 February 2019 and removed after harvest and then the same mulch was installed in the 'Rosy Glow' trials on 28 March 2019.

Data collection. The PAR was measured using a PAR/LAI ceptometer, ACCUPAR (Decagon Devices, Inc. model LP-80, Pullman, Washington, USA) on two cloudless days (12 and 22 April 2019) between 11:00 a.m. and 14:00 p.m. on the farm Paardekloof. Measurements were taken for each reflective mulch and the untreated control for both trials as illustrated in Fig. 2. The maximum incident light was measured in the center of the plot at the tagged trees by holding the device perpendicularly 1 m above the ground facing upwards (a). Another measurement was taken in the same way, except holding the device perpendicularly facing downwards to determine the maximum reflected light (b). To determine the maximum percentage of light reflected, (b) is divided by (a) and multiplied by 100 (Fig. 2). A further two measurements were taken on both the east and west sides of the strip by holding the device 30 cm above the ground and touching the tree trunk in a perpendicularly downward position. This coincides with where the lowest fruit in the tree canopy occurred and serves as an indication of actual reflected PAR reaching the lower canopy fruit.

In addition, the following data were collected in all four trials. In 'Fuji' harvest commenced five weeks after the mulches were installed on 26 March 2019, while in 'Rosy Glow' harvest commenced on 24 April 2019 four weeks after installation of the mulches. The two selected trees per plot were strip picked, keeping fruit harvested from the top east, bottom east, top west and bottom west canopy positions separate. The upper and lower canopy positions were separated at shoulder height. Harvested fruit were taken to the laboratory at Stellenbosch University immediately where evaluations were done. All fruit were individually weighed, and the diameter and height measured using an electronic caliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan). The color of each fruit was determined by scoring the percentage blush coverage, the blush intensity according to the Pink Lady™ color chart (Paper 1) and the UNIFRUCO Fuji apple color chart (Paper 1) for 'Rosy Glow' and 'Fuji', respectively. Ground color of each fruit was also determined according to the UNIFRUCO color chart for apples and pears (Paper 1). A further subset of 30 fruit was taken for maturity indexing. Starch breakdown was determined using the UNIFRUCO starch conversion chart (Paper 1) as well as fruit firmness using the GÜSS texture analyzer (Güss electronic model GS 20, Strand, South Africa).

Statistical analysis. All data were analyzed using SAS enterprise guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA) using a three-way factorial analysis with reflective mulch treatment (product), canopy position (position) and -direction (direction) as factors. In cases where the F-statistic indicated a significance at P<0.05 level, the pairwise t-test was used to obtain the Least Significant Difference (LSD).

Results

PAR measurements. Photosynthetically active radiation measurements taken in the middle of the uncovered 'Rosy Glow' orchard inter-row as a reference of the maximum percentage of PAR reflected increased significantly from 7.1% (untreated control) to 53.2% and 53.0% for Lumilys® and ColorIt, respectively (Table 1). Also, under the permanent shade net, PAR was increased by both reflective mulches to 52.2 and 44.4%, respectively, for Lumilys® and ColorIt compared to the 6.3% in the untreated control (Table 2). Under net, the Lumilys®, however, improved the reflected light significantly more than ColorIt. PAR readings taken 30 cm above the orchard floor and in the area from the trunk, to 1 m from the trunk, on either side of the row indicated that both reflective mulches significantly increased reflected light in the eastern and western side of the lower canopy compared to the grassed alley. The two mulches did not differ significantly from each other and improved light on both sides of the trees in the uncovered orchard, but in the net-covered orchard, Lumilys® improved reflected PAR more on the western side of the canopy than ColorIt.

'Fuji': Fruit quality. No significant interactions were found in the Fuji' trial without draped net between factors in average percentage blush, percentage fruit with 50% blush or more, average blush intensity or ground color (Table 3). Although the percentage blush coverage of fruit was not statistically different between the control and either of the two mulches, both mulches significantly increased the percentage of fruit with 50% blush coverage or more by ca. 9%. Blush color intensity as well as ground color were not affected by mulches when compared to the control. The percentage blush and fruit with more than 50% blush were higher in the upper canopy compared to the lower canopy and higher on the western than the eastern side of the trees. The average blush color intensity was higher in fruit from the lower canopy compared to the upper canopy (Table 3). Fruit ground color was not affected by canopy position (upper vs lower or east vs. west) (Table 3).

Fruit size (weight, height, and diameter) and firmness did not differ statistically between the control, Lumilys® and ColorIt treatments in the open 'Fuji' trial (Table 4). However, starch breakdown was significantly lower in fruit harvested from ColorIt treated trees than that of Lumilys® and the control, which were statistically similar. Starch breakdown in fruit from the upper canopy and from the western side of the tree was less advanced than the lower canopy and eastern side, respectively (Table 4).

In the 'Fuji' trees under draped net, the percentage blush of individual fruit was significantly increased by both Lumilys® and ColorIt compared to the grassed alley (Table 5). The two products did not differ from each other. Fruit from the upper canopy also displayed on average a higher blush percentage than fruit from the lower canopy, while fruit from the western side of the trees did not differ significantly from that on the eastern side. ColorIt increased blush intensity compared to control and Lumilys® fruit and fruit in the upper canopy also displayed higher blush intensity than in the lower canopy (Table 5). Fruit ground color was greener in Lumilys® and control fruit compared to ColorIt fruit, and so was fruit from the western canopy compared to the eastern canopy (Table 5). A significant interaction was observed between the product and the position of fruit on the tree regarding the percentage of fruit with 50% or more blush coverage (Table 6). A higher percentage fruit with more than 50% blush coverage was found in the upper canopy, although not significantly so for Lumilys® or Colorit.

Fruit weight and height were not significantly affected by Lumilys® compared to the control, whereas an increase in both these parameters was observed following installation of ColorIt (Table 7). The increase in weight in Colorit treated fruit did not differ significantly compared to the weight of Lumilys® treated fruit. Both mulches resulted in an increased fruit diameter. Fruit size was bigger on the eastern side than the western side of the tree canopy. Fruit firmness was not affected by either mulch treatment, but upper and western canopy fruit were firmer than lower and eastern canopy fruit, respectively. Starch breakdown of the control and ColorIt fruit was statistically similar, whereas Lumilys® fruit had lower starch breakdown (Table 7). Upper and western canopy fruit also had less starch breakdown compared to lower and eastern canopy fruit, respectively.

'Rosy Glow': Fruit quality. Significant product and position interaction occurred in average percentage red blush of fruit, percentage Pink Lady™ (fruit with more than 40% blush coverage, market-dependent) as well as blush color intensity in the open trial (Table 8).

Generally, a higher percentage blush coverage was found in the upper canopy. Interestingly, the blush percentage of the fruit from the upper canopy was also increased significantly by both Lumilys® and ColorIt compared to the control. Fruit from the western side had slightly more blush coverage (76.6%) compared to fruit from the eastern side (74.4%). The percentage fruit classed as Pink Lady™ was high in all upper canopies and in the lower canopy, except in the lower canopy control trees (Table 8). Blush color intensity was higher in the upper canopy for the grassed alley, Lumilys® and ColorIt, but the difference between the upper and lower canopy was greater in the grassed alleys (Table 8). Lumilys® and ColorIt improved blush intensity compared to the control in both the upper and lower canopy.

Fruit size as well as ground color were not affected by reflective mulch or tree canopy direction in the open orchard (Table 9). Fruit from the upper canopy were smaller than fruit from the lower canopy. Lumilys® slightly decreased fruit firmness compared to the control and ColorIt. Significant interaction was observed between position and direction for the percentage starch breakdown of fruit (Table 10). The starch breakdown was consistently less in lower canopy positions, and significantly more so on the eastern side of the canopy compared to the western side. There were no significant differences noted for the starch breakdown between products and the untreated control.

There was significant interaction between the mulching treatment and fruit position for average percentage blush coverage, percentage Pink Lady™ and the blush color intensity of fruit grown under permanent nets, however no significant difference due to product was observed for any of the beforementioned parameters (Table 11). Both the percentage blush and the percentage fruit classified as Pink Lady™ apples in the lower canopy was increased by Lumilys® and ColorIt to the extent that it did not differ significantly from the upper canopy fruit. The control lower canopy fruit had a significantly lower blush and Pink Lady™ percentage. The blush color intensity of fruit from upper and lower positions differed significantly for the control as well as both reflective mulches, but more so in the control.

Fruit size did not differ significantly among mulching treatments or between the eastern and western sides of the tree canopy. Fruit size was greater in the lower than the upper tree canopy (Table 12). Slightly decreased fruit firmness was observed in fruit from the Lumilys® reflective mulch and even more so from ColorIt compared to the control. Fruit from the lower tree canopy were less firm than those from the upper tree canopy. Both Lumilys® and ColorIt mulches resulted in fruit with greener ground color compared to the untreated control (Table

12). An interaction between the position and fruit direction within the canopy was found in starch breakdown. Fruit from the lower, eastern canopy had more advanced starch breakdown (Table 13). There was, however, no significant difference among mulches and the untreated control.

Yield. In the 'Fuji' trial without draped net, the yield and yield efficiency were higher on the eastern side of the tree canopy than on the western side but did not differ significantly with mulching treatment or canopy position (Table 14). No significant differences were found in yield or yield efficiency in the 'Fuji' trial under draped net. In the 'Rosy Glow' trial without net, the yield was slightly higher in the lower tree canopy but did not differ with reflective mulch treatment or between the eastern or western side of the tree. Yield efficiency only differed with the reflective mulch treatment, where Lumilys® treated trees had a slightly lower yield efficiency compared to the grassed alley and ColorIt (Table 15). In the 'Rosy Glow' trial under permanent shade net, the yield was slightly higher on the western side of trees compared to the eastern side but did not differ with reflective treatment or between the upper or lower canopies (Table 15). Yield efficiency however interacted between the reflective mulch product and position in the canopy of trees under net (Fig. 3), and a slight decrease in yield efficiency is evident in trees where Lumilys® was installed compared to the control and ColorIt.

Discussion

PAR was increased 5.0 and 7.6-fold by Lumilys® under permanent shade net on the western and eastern sides respectively, and 4.1 and 6.9-fold by ColorIt in the same positions of the interrow at 30 cm above the ground. In the case where no shade net was present, Lumilys® increased PAR 4.9 and 5.9 times for the west and east side, respectively, and ColorIt increased PAR 4.8 and 4.9-fold for west and east side respectively, 30 cm from the ground. PAR measurements were taken from 11 a.m. to 2 p.m., thus the east side displayed higher PAR readings in all cases due to the position of the sun at that time of day. This is in concurrence with the findings of Privé et al. (2008) who reported a 5 to 9-fold increase in PAR into the lower canopy by Extenday™, measured over the course of the growing season on 'Gala' apples. Interestingly, their findings also indicate a significant increase in PAR not only into the middle and inner canopy, but also above the tree canopy. The overall PAR increase described by Privé et al. (2008) had a positive effect on the photosynthetic efficiency of the orchard. This is a valuable finding, since sink demands of fruit are especially taxing on tree assimilate availability during the growing season. A similar effect on photosynthesis was reported by Leão de Sousa and Sánchez (2020).

'Fuji'. Sufficient light is essential for red color development in apples. Shade netting significantly reduces the irradiance reaching the tree canopy and therefore the fruit. Weber et al. (2019) found that a crystalline hail net can lead to a 21.8% reduction in light on overcast days and 17.5% reduction on sunny days. The increase in percentage blush coverage of 6.4% and 9.2% by Lumilys® and ColorIt, respectively relative to the control apples under 20% white draped net, compared to the smaller increase of circa 3% for both mulches relative to the control without net illustrates the importance of light for fruit color development and the positive influence of reflective mulches, especially under shade netting. The data also highlights the influence that fruit position has on color development, showing that fruit from the upper canopy as well as the western direction tend to color better than their counterparts. Overall, both reflective mulches increased the number of fruit with more than 50% blush coverage, especially so in the lower canopy. This is due to the increase in diffuse light reflected from the mulch, as shown by PAR measurements (Table 1 and 2), reaching fruit that would usually receive insufficient light, since most incident PAR would be absorbed, not reflected, by the cover crop in the orchard interrow.

A slight increase in red color intensity was also seen in fruit where reflective mulch was installed next to trees covered with draped net. This concurs with the results of both Smrke et al. (2010) and Leão de Sousa and Sánchez (2019) who noted increased red color intensity on 'Tipo' persimmon and 'Fuji' apples, respectively, using reflective mulching for approximately one month before harvest, although the increase in red color in persimmon is attributed to an increase in carotenoids.

Fruit firmness and size were not affected by the mulching treatment where trees were not covered by draped net. Fruit from trees under draped net were slightly larger when the two mulches were used compared to the control fruit. This could be due to increased light availability causing an upregulation in photosynthetic activity, allowing greater carbohydrate availability to fruit (Baïram et al., 2019). Fruit form the eastern side of the tree canopy under net were consistently larger than fruit from the western side. Fruit firmness was also lower in both fruit from the lower canopy and fruit from the eastern side of the canopy. This is supported by higher starch breakdown in fruit from the same canopy positions, indicating that fruit from the lower canopy and eastern side were more mature than their counterparts. Fruit without draped net displayed starch breakdown of 49.1% and 41.6% for Lumilys® and ColorIt respectively, compared to 52.2% for the untreated control. Fruit covered by draped net showed a similar trend where starch breakdown was 36.8% and 44.0% for Lumilys® and Colorit respectively, compared to 43.4% for the control. This concurs with results obtained by Schuhknecht et al. (2018) where Extenday™ and Lumilys® reflective mulches were installed seven weeks before harvest in a 'Braeburn' orchard. Overbeck et al. (2013), on the other hand, reported that reflective mulch installed five weeks before harvest in 'Gala Mondial' significantly enhanced the starch breakdown of fruit. However, other maturity parameters such as fruit firmness were not affected in the same trial.

'Rosy Glow'. For 'Rosy Glow' apples to be graded as class one and sold under the tradename Pink Lady™, a blush coverage of at least 40% is required (Pink Lady, 2019). Blush color is therefore a major determinant of potential profit in the market, both locally and for export. Often, fruit found in the lower canopy lack adequate blush coverage due to a lack of light exposure (Weber et al., 2019). This causes a large part of fruit from trees to be downgraded due to insufficient color, even though fruit quality otherwise is excellent. In these trials, a significant increase in percentage blush coverage of lower canopy fruit was observed where reflective mulch was installed in trees covered by shade net as well as uncovered trees compared to controls. Fruit from the lower canopy from both reflective mulches had a blush percentage and class one pack-out similar to control fruit from the upper canopy. This is due to increased light exposure from reflective mulches resulting in upregulation of anthocyanin synthesis (Weber et al., 2019). Leão de Sousa and Sánchez (2020) reported similar results where a UV stabilized white polypropylene reflective film was installed on 'Fuji' apples 22 days before harvest and increased PAR from 9.1% for the control to 53.6% in the middle of the orchard alley. Their trial indicated that fruit from the inside canopy of mulched trees had similar red color to fruit located in the outside canopy positions from trees without reflective film. Weber et al (2019) found that fruit from sun-exposed positions on the tree canopy displayed no difference in red color development between areas mulched with Lumilys® and uncovered control areas. Fruit from the lower and inside canopy of mulched areas, however, had distinguishably enhanced red coloration. This is attributed to increased diffuse light from the reflective mulch upregulating anthocyanin synthesis in fruit. Schuhknecht et al. (2018) compared both Extenday™ and Lumilys® reflective mulches to an uncovered control and found that even in a year with conditions that favored fruit coloration, reflective mulching

increased the percentage of class one 'Braeburn' apples by 44% and 31% for Extenday™ and Lumilys®, respectively, compared to the grassed alley control.

Interaction was found between the product and position of fruit on the canopy for blush intensity with and without shade net. The blush intensity of fruit from the upper canopy did not differ significantly from the control, irrespective of treatment, except in the case of ColorIt fruit in the open trial, which had significantly greater red color intensity than the control and Lumilys®. Fruit from the lower canopy of both Lumilys® and ColorIt consistently had higher blush intensity than that of the control, although not significantly so under netting. This further highlights the influence that reflective mulching has on color development of lower canopy fruit. Ground color of apples under permanent shade net was slightly greener for both Lumilys® and ColorIt compared to the control, although this difference is very small and of no biological consequence.

Neither of the two mulches influenced fruit size or starch breakdown, although fruit from the upper tree canopy were generally larger in size and less mature than fruit from lower canopy positions. It appears the mulches slightly decreased average fruit firmness, except for ColoIt in the open trial. However, in both cases with and without shade netting, fruit firmness was well above the Pink Lady™ minimum requirement of 6.5 $kg \cdot cm^{-2}$. Overbeck et al. (2013) found that various reflective mulches, including Extenday™ and Daybright™, improved fruit coloration due to increased anthocyanin levels, while fruit firmness, size and yield was unaffected. Since 'Rosy Glow' is harvested based on a minimum color requirement, the increase in percentage blush by mulch installation of especially lower and inner canopy fruit, which are usually more mature than upper, outer canopy fruit, will allow these fruit to be harvested earlier, when at optimum maturity. This will allow for longer storage and reduce the risk of disorders such as diffuse internal browning.

General. In terms of installation ease and general remarks, ridging, which is a common practice in South African orchards, posed some difficulty to installation of the mulches. Since the reflective mulch is secured to tree trunks by elastic chords, ridges cause the mulch to be elevated higher than the recommended 10 cm from the ground. To overcome this problem, however, sandbags were placed at approximately 3 m intervals, which ensured that the mulch was still appropriately elevated from the orchard floor. Moreover, ColorIt is installed by fixing the mulch to fruit tree trunks using gator clips, where Lumilys® is installed in a similar way but using hooks attached to elasticated chord which pierce the woven mulch. Both methods of installation are equally effective and non-damaging to the fabric since the woven nature of the fabric allows fibers to shift to create an opening rather than the fibers tearing. Long term, however, the hooks may cause some damage to the fabric if reinserted over many seasons, since they are quite sharp and may cause accidental tears of fibers. In a practical sense, the hook system is more easily replicable than the gator clips, if for instance there is theft or loss of fastenings. All in all, it is up to the producer which system is preferred, as on a performance level the two products are quite similar.

Cover crops in the orchard alley rows were undisturbed by both mulches. Much of this is probably since water can permeate freely through both weaved reflective mulches and soil ecology is not disrupted. Privé et al (2008) noted that Extenday™ installed from full bloom up until three weeks after harvest in 2003 and 2004 resulted in an average increase in soil moisture and decrease of 2.1 – 2.5 ˚C in soil temperature up to a depth of 10 cm. Most of the soil moisture and temperature differences were observed early in the growing season and would therefore not have a prolonged effect on soil microclimate. Cover crops that were unaffected are a good indication that the soil microclimate and biology were undisturbed by the mulches. Furthermore, although mulches are quite expensive, they can be used for various cultivars within a season and can last for multiple seasons. Not only does the reusability of the mulch justify the cost, but also the economic benefit from increased color development. Between the two mulches, both performed similarly in increasing red color development of problematic areas within the canopy without affecting fruit maturity.

Conclusion

Reflective mulching shows potential for increasing red color development in bi-color apples, especially fruit from problematic areas such as the lower canopy positions. Visible pack-out differences between the control and reflective mulches were seen even though the season generally seemed to have been favorable for red color development. Both reflective mulches successfully increased reflected PAR reaching the tree canopy, especially the lower tree canopy. Regarding color development, fruit quality and maturity parameters, Lumilys® and ColorIt performed equally. Both fabrics are available in various widths, making the mulch customizable to specific orchard specifications. High cost of reflective mulches can be justified by the reusability between cultivars and for multiple seasons, as well as the economic benefit from increased class one pack-outs and export income because of increased red color development. Mulch installation also results in more uniform color development throughout the tree canopy, allowing problematic fruit such as lower and inner fruit that would have otherwise been left on the tree too long for color development, to be harvested at optimum maturity. This should improve the long-term storage potential of a crop from the orchard and reduces the risk of internal disorders, further decreasing fruit- and therefore profit loss.

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Fig. 1. (a) Photograph of 'Fuji' open orchard at Graymead Farm, Vyeboom with reflective mulch installed. (b) 'Fuji' orchard at Graymead Farm, Vyeboom with reflective mulch installed next to draped net. (c) Photograph of control 'Cripps' Pink' trees at Paardekloof Farm, Ceres. (d) Photograph of control 'Cripps' Pink' apples at Paardekloof Farm, Ceres with ColorIt installed. (e) Photograph of control 'Cripps' Pink' apples at Paardekloof Farm, Ceres. (f) Photograph of control 'Cripps' Pink' apples at Paardekloof Farm, Ceres with Lumilys® installed. (Photographs: Natalie Steyn)

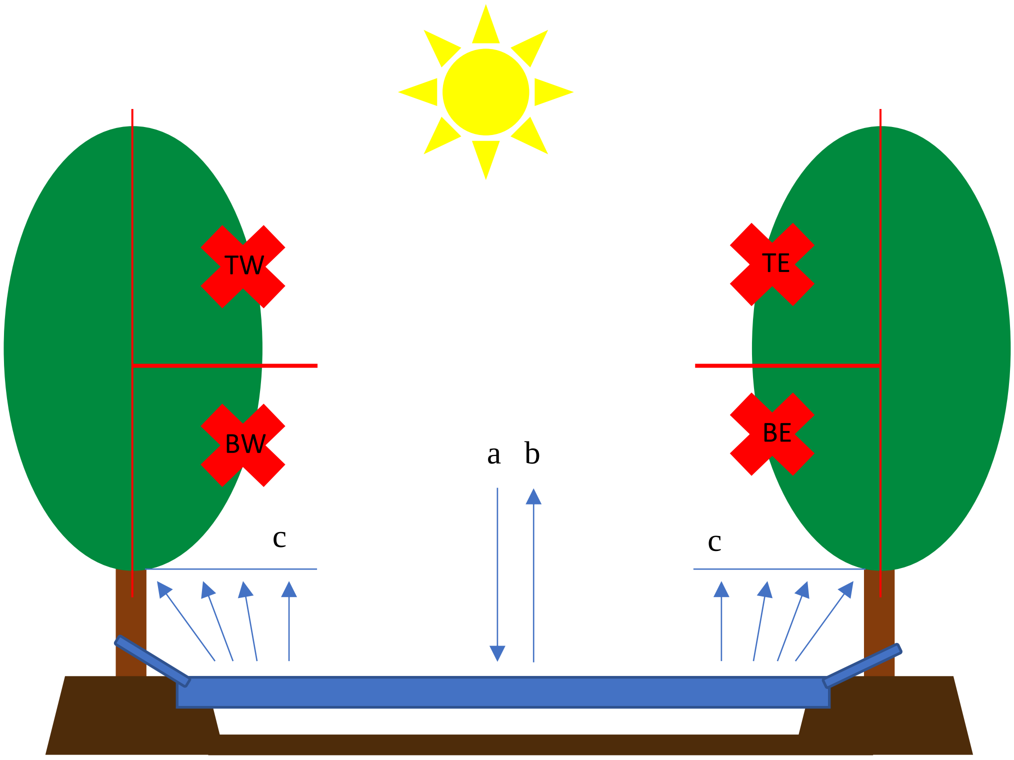

Fig. 2. Illustration of sampling quadrants and PAR measurements taken between 11:00 and 14:00 on 12 and 22 April 2019 at Paardekloof, Ceres, South Africa. Mulch was approximately 10 cm from the ground. (TW) indicates the top west sampling position, (BW) bottom west sampling quadrant, (TE) top east sampling position and (BE) the bottom eastern sampling quadrant. (a) measurement taken with the device facing towards the sun perpendicular to the ground in the middle of the alley 1 m above the ground. This indicates **total incident light**. (b) measurement taken with device facing towards the ground and perpendicular to the ground. Again, measurement was taken in the middle of the interrow at a height of 1 m. This is the **maximum PAR that is reflected** from the reflective mulch. (c) measurements taken at the east and west sides of the alley. Probe was held perpendicularly to the ground, with sensors facing perpendicularly downwards at a height of 30 cm and touching the tree trunk. The probe, which is 86.5 cm in length, which contains 80 sensors, measures the average PAR reflected over that length, giving the average **PAR reaching bottom hanging fruit**. To determine the maximum percentage of light reflected, (b) is divided by (a) and multiplied by 100.

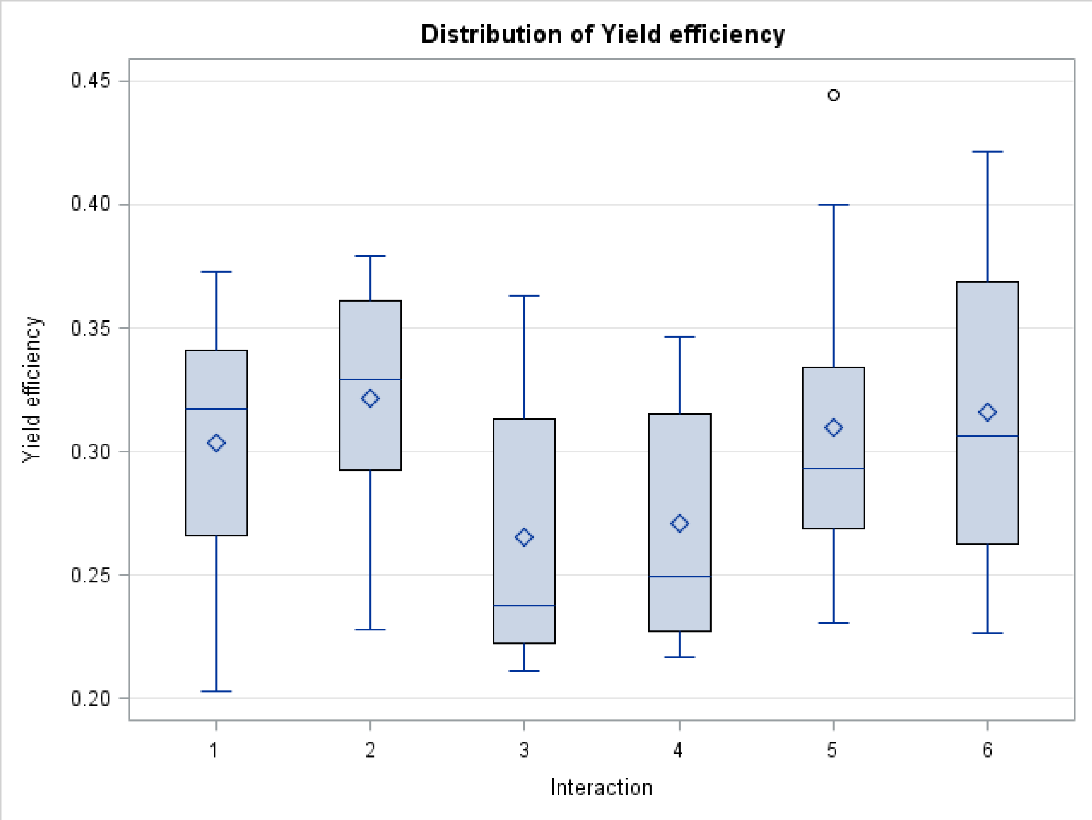

Fig. 3. Effect of reflective mulch product and position (interaction) in the tree canopy on yield efficiency of 'Rosy Glow' apples under permanent net at Paardekloof. (1) indicates the upper position of the control canopy, (2) the lower position of the control canopy. (3) indicates fruit from the upper canopy position of Lumilys and (4) the lower canopy fruit from Lumilys mulch. (5) indicates the upper position of the ColorIt mulched tree canopy and (6) the lower position of the ColorIt canopy.

Table 1. Average photosynthetically active radiation (PAR) readings taken between 11:00 and 14:00 on 12 and 22 April 2019 in a ‘Rosy Glow’ open orchard at Paardekloof, Ceres, South Africa.

	Maximum percentage of incident light reflected in the center of the treatment plot		Average Reflected PAR on the western side of the tree ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		Average Reflected PAR in the eastern side of the tree ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	
Control	7.2	b	43.6	b	90.5	b
Lumilys®	53.2	a	212.5	a	526.8	a
ColorIt	53.0	a	208.1	a	446.5	a
Significance level	***<.0001***		***<.0001***		***<.0001***	
LSD 5%	*4.9*		*56.5*		*107.4*	

Table 2. photosynthetically active radiation (PAR) readings taken between 11:00 and 14:00 on 12 and 22 April 2019 in a ‘Rosy Glow’ orchard with permanent shade net. Data is the average values measured on both days at Paardekloof, Ceres, South Africa.

	Maximum percentage of incident light reflected in the center of the treatment plot		Average Reflected PAR on the western side of the tree ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		Average Reflected PAR in the eastern side of the tree ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	
Control	6.3	c	27.1	c	57.6	b
Lumilys®	52.2	a	135.4	a	436.1	a
ColorIt	44.4	b	111.7	b	396.3	a
Significance level	***<.0001***		***<.0001***		***<.0001***	
LSD 5%	*4.9*		*20.8*		*51.8*	

Table 3. Effect of reflective mulches on fruit color of ‘Fuji’ apples without draped net at Graymead, Grabouw (2019)

		Percentage blush		Percentage fruit with 50% blush or more		Blush color intensity*		Fruit ground color**	
Product									
	Control	53.41	ns	68.54	b	4.65	ns	2.16	ns
	Lumilys®	56.30		77.58	a	4.42		2.20	
	ColorIt	56.46		77.46	a	4.62		2.13	
Position									
	Upper canopy	58.93	a	81.67	a	4.01	b	2.16	ns
	Lower canopy	51.85	b	67.39	b	5.11	a	2.17	
Direction									
	West	57.90	a	81.53	a	4.38	ns	2.20	ns
	East	52.89	b	67.53	b	4.75		2.13	
Significance level									
	Product	*0.1041*		***0.0353***		*0.5794*		*0.6180*	
	Position	***<.0001***		***<.0001***		***<.0001***		*0.9362*	
	Direction	***0.0003***		***<.0001***		*0.0610*		*0.2757*	
	*Product*Position*	*0.0844*		*0.1257*		*0.5170*		*0.8859*	
	*Product*Direction*	*0.8942*		*0.6995*		*0.9253*		*0.9918*	
	*Position*Direction*	*0.2823*		*0.3855*		*0.7016*		*0.9398*	
	*Product*Position*Direction*	*0.1939*		*0.1685*		*0.2896*		*0.7447*	

*Blush color intensity is measured using the UNIFRUCO color chart for ‘Fuji’ apples and ranges from a scale of 1 to 12, 1 being the most intense and 12 being the least intense red color.

**Ground color is measured using the UNIFRUCO color chart for apples and pears and ranges from a scale of 0.5 to 5, 0.5 indicating green and 5 indicating yellow ground color.

Table 4. Effect of reflective mulches on size and maturity of 'Fuji' apples without draped net at Graymead, Grabouw (2019)

	Fruit weight (g)		Fruit height (mm)		Fruit diameter (mm)		Fruit firmness (kg)		Starch breakdown (%)	
Product										
Control	123.36	ns	53.61	ns	67.18	ns	7.81	ns	52.15	a
Lumilys®	126.15		53.83		68.04		7.75		49.07	a
ColorIt	120.81		53.32		66.88		7.90		41.55	b
Position										
Upper canopy	124.63	ns	53.78	ns	67.61	ns	7.84	ns	45.53	b
Lower canopy	122.26		53.39		67.12		7.80		49.65	a
Direction										
West	120.97	ns	53.06	b	67.06	ns	7.87	ns	42.26	b
East	125.92		54.11	a	67.67		7.77		52.92	a
Significance level										
Product	*0.2458*		*0.5966*		*0.1241*		*0.0897*		***<.0001***	
Position	*0.3605*		*0.3426*		*0.3072*		*0.5477*		***0.0222***	
Direction	*0.0590*		***0.0127***		*0.2017*		*0.0856*		***<.0001***	
*Product*Position*	*0.8637*		*0.8216*		*0.7981*		*0.5616*		*0.8746*	
*Product*Direction*	*0.9029*		*0.7651*		*0.9378*		*0.2433*		*0.4292*	
*Position*Direction*	*0.1894*		*0.2101*		*0.1479*		*0.4290*		*0.8355*	
*Product*Position*Direction*	*0.7686*		*0.6684*		*0.7437*		*0.9925*		*0.6856*	

Table 5. Effect of reflective mulches on fruit color of 'Fuji' apples under draped net at Graymead, Grabouw (2019)

		Percentage blush		Blush color intensity*		Fruit ground color**	
Product							
	Control	55.66	b	4.09	a	2.48	b
	Lumilys®	62.03	a	3.72	a	2.57	b
	ColorIt	64.84	a	3.10	b	2.82	a
Position							
	Upper canopy	66.95	a	2.66	b	2.60	ns
	Lower canopy	54.74	b	4.62	a	2.64	
Direction							
	West	61.03	ns	3.81	ns	2.56	b
	East	60.65		3.47		2.68	a
Significance level							
	Product	***<.0001***		***0.0004***		***<.0001***	
	Position	***<.0001***		***<.0001***		*0.4788*	
	Direction	*0.8030*		*0.0847*		***0.0205***	
	*Product*Position*	*0.4097*		*0.7837*		*0.9182*	
	*Product*Direction*	*0.5848*		*0.6369*		*0.8437*	
	*Position*Direction*	*0.8123*		*0.7826*		*0.0693*	
	*Product*Position*Direction*	*0.9424*		*0.7113*		*0.7676*	

*Blush color intensity is measured using the UNIFRUCO color chart for 'Fuji' apples and ranges from a scale of 1 to 12, 1 being the most intense and 12 being the least intense red color.
**Ground color is measured using the UNIFRUCO color chart for apples and pears and ranges from a scale of 0.5 to 5, 0.5 indicating green and 5 indicating yellow ground color.

Table 6. Effect of reflective mulches on the percentage of 'Fuji' apples with a blush percentage of 50% or greater under draped net at Graymead, Grabouw (2019)

		Percentage fruit with 50% blush or more	
Direction			
	West	79.85	ns
	East	80.94	
Interaction (Product*Position)			
	Control Upper canopy	87.50	a
	Control Lower canopy	55.16	b
	Lumilys® Upper canopy	89.50	ab
	Lumilys® Lower canopy	79.57	ab
	ColorIt Upper canopy	93.38	a
	ColorIt Lower canopy	77.26	ab
Significance level			
	Product	*<.0001*	
	Position	*<.0001*	
	Direction	*0.6906*	
	*Product*Position*	***0.0043***	
	*Product*Direction*	*0.5672*	
	*Position*Direction*	*0.3105*	
	*Product*Position*Direction*	*0.8398*	

Table 7. Effect of reflective mulches on size and maturity of 'Fuji' apples under draped net at Graymead, Grabouw (2019)

	Fruit weight (g)		Fruit height (mm)		Fruit diameter (mm)		Fruit firmness (kg)		Starch breakdown (%)	
Product										
Control	115.84	b	51.92	b	66.19	b	8.09	ns	43.44	a
Lumilys®	122.03	ab	52.99	b	67.72	a	8.08		36.82	b
ColorIt	127.20	a	54.56	a	68.25	a	8.30		44.02	a
Position										
Upper canopy	122.64	ns	53.37	ns	67.64	ns	8.28	a	37.76	b
Lower canopy	120.74		52.95		67.14		8.03	b	45.09	a
Direction										
West	116.12	b	52.30	b	66.36	b	8.25	a	37.52	b
East	127.26	a	54.02	a	68.41	a	8.06	b	45.33	a
Significance level										
Product	***0.0126***		***0.0012***		***0.0167***		*0.0818*		***0.0091***	
Position	*0.5322*		*0.4558*		*0.4040*		***0.0069***		***0.0007***	
Direction	***0.0005***		***0.0031***		***0.0010***		***0.0365***		***0.0003***	
*Product*Position*	*0.5102*		*0.7398*		*0.4612*		*0.9831*		*0.3327*	
*Product*Direction*	*0.5322*		*0.5672*		*0.5002*		*0.2265*		*0.5978*	
*Position*Direction*	*0.2377*		*0.2756*		*0.2860*		*0.5425*		*0.9549*	
*Product*Position*Direction*	*0.3403*		*0.4626*		*0.4040*		*0.8471*		*0.7353*	

Table 8. Effect of reflective mulches on fruit color and maturity of 'Rosy Glow' apples without net at Paardekloof, Ceres (2019)

	Percentage blush		Percentage fruit with 40% blush or more		Blush color intensity*	
Direction						
West	76.60	ns	96.34	ns	9.81	ns
East	74.73		95.41		9.75	
Interaction (Product*Position)						
Control Upper canopy	74.66	bc	97.47	a	10.11	b
Control Lower canopy	60.48	d	86.88	b	8.30	d
Lumilys® Upper canopy	81.86	a	98.94	a	10.27	b
Lumilys® Lower canopy	73.34	c	95.55	a	9.16	c
ColorIt Upper canopy	85.04	a	99.12	a	10.90	a
ColorIt Lower canopy	77.52	b	97.30	a	9.94	b
Significance level						
Product	*<.0001*		<.0001		<.0001	
Position	*<.0001*		<.0001		<.0001	
Direction	***0.0438***		0.3577		0.6126	
*Product*Position*	***0.0311***		**0.0016**		**0.0099**	
*Product*Direction*	*0.9325*		0.6617		0.7506	
*Position*Direction*	*0.0782*		0.1358		0.9401	
*Product*Position*Direction*	*0.7694*		0.4233		0.5057	

*Blush color intensity is measured using the Pink Lady™ color chart and ranges from a scale of 1 to 12, 1 being the least intense and 12 being the most intense red color.

Table 9. Effect of reflective mulches on fruit size and maturity of 'Rosy Glow' apples without net at Paardekloof, Ceres (2019)

	Fruit weight (g)		Fruit height (mm)		Fruit diameter (mm)		Fruit firmness (kg)		Ground color*	
Product										
Control	166.01	ns	65.44	ns	74.41	ns	9.31	a	3.46	ns
Lumilys®	167.36		65.71		74.59		9.03	b	3.43	
ColorIt	170.54		66.45		75.22		9.32	a	3.44	
Position										
Upper canopy	162.02	b	64.96	b	73.81	b	9.25	ns	3.41	ns
Lower canopy	173.91	a	66.77	a	75.68	a	9.18		3.48	
Direction										
West	168.26	ns	65.97	ns	74.62	ns	9.21	ns	3.45	ns
East	167.67		65.76		74.87		9.23		3.43	
Significance level										
Product	*0.3824*		*0.1162*		*0.2910*		***0.0002***		*0.7474*	
Position	***<.0001***		***<.0001***		***<.0001***		*0.2282*		*0.0881*	
Direction	*0.8290*		*0.5908*		*0.5719*		*0.8013*		*0.5484*	
*Product*Position*	*0.3252*		*0.2797*		*0.3756*		*0.5203*		*0.3465*	
*Product*Direction*	*0.2978*		*0.5140*		*0.5147*		*0.1282*		*0.8594*	
*Position*Direction*	*0.7053*		*0.8865*		*0.6128*		*0.7770*		*0.5348*	
*Product*Position*Direction*	*0.9538*		*0.9623*		*0.9205*		*0.8219*		*0.6584*	

*Ground color is measured using the UNIFRUCO color chart for apples and pears and ranges from a scale of 0.5 to 5, 0.5 indicating green and 5 indicating yellow ground color.

Table 10. Effect of reflective mulches on starch breakdown of 'Rosy Glow' apples without net at Paardekloof, Ceres (2019)

		Percentage starch breakdown	
Product			
	Control	50.52	ns
	Lumilys®	49.04	
	ColorIt	50.88	
Interaction (Postion*Direction)			
	West Upper canopy	47.92	c
	West Lower canopy	50.86	b
	East Upper canopy	47.92	c
	East Lower canopy	54.94	a
Significance level			
	Product	*0.2587*	
	Position	*<.0001*	
	Direction	*0.1181*	
	*Product*Position*	*0.5767*	
	*Product*Direction*	*0.9689*	
	*Position*Direction*	***0.0097***	
	*Product*Position*Direction*	*0.3016*	

Table 11. Effect of reflective mulches on fruit color and maturity of 'Rosy Glow' apples under permanent net at Paardekloof, Ceres (2019)

	Percentage blush		Percentage fruit with 40% blush or more		Blush color intensity*	
Direction						
West	71.04	ns	93.72	ns	9.04	ns
East	70.95		95.47		9.18	
Interaction (Product*Position)						
Control Upper canopy	75.73	a	97.33	a	10.05	a
Control Lower canopy	59.41	b	87.25	b	8.03	d
Lumilys® Upper canopy	76.04	a	97.26	a	9.46	ab
Lumilys® Lower canopy	69.29	a	94.00	a	8.75	c
ColorIt Upper canopy	75.08	a	96.90	a	9.47	ab
ColorIt Lower canopy	70.41	a	94.83	a	8.91	bc
Significance level						
Product	*0.0013*		0.0405		0.8222	
Position	*<.0001*		0.0001		<.0001	
Direction	*0.9430*		0.1686		0.4663	
*Product*Position*	***0.0007***		**0.0246**		**0.0047**	
*Product*Direction*	*0.6295*		0.7978		0.8442	
*Position*Direction*	*0.9092*		0.7025		0.7086	
*Product*Position*Direction*	*0.8657*		0.9830		0.6385	

*Blush color intensity is measured using the Pink Lady™ color chart and ranges from a scale of 1 to 12, 1 being the least intense and 12 being the most intense red color.

Table 12. Effect of reflective mulches on fruit size and maturity of 'Rosy Glow' apples under permanent net at Paardekloof, Ceres (2019)

	Fruit weight (g)		Fruit height (mm)		Fruit diameter (mm)		Fruit firmness (kg)		Ground color*	
Product										
Control	181.06	ns	68.25	ns	77.42	ns	9.22	a	3.37	a
Lumilys®	177.53		68.19		77.30		8.98	b	3.18	b
ColorIt	178.13		68.37		77.31		8.84	c	3.11	b
Position										
Upper canopy	172.11	b	67.03	b	76.37	b	9.10	a	3.21	ns
Lower canopy	185.70	a	69.51	a	78.31	a	8.92	b	3.24	
Direction										
West	179.12	ns	68.32	ns	77.34	ns	9.01	ns	3.23	ns
East	178.70		68.22		77.35		9.01		3.21	
Significance level										
Product	*0.5978*		*0.9454*		*0.9733*		***<.0001***		***<.0001***	
Position	***<.0001***		***<.0001***		***0.0002***		***0.0005***		*0.4368*	
Direction	*0.8898*		*0.8317*		*0.9718*		*0.9880*		*0.6112*	
*Product*Position*	*0.7541*		*0.8333*		*0.7288*		*0.7063*		*0.0955*	
*Product*Direction*	*0.8074*		*0.7578*		*0.9656*		*0.9002*		*0.8550*	
*Position*Direction*	*0.8650*		*0.7978*		*0.8208*		*0.9805*		*0.5823*	
*Product*Position*Direction*	*0.3930*		*0.4496*		*0.3706*		*0.1573*		*0.9271*	

*Ground color is measured using the UNIFRUCO color chart for apples and pears and ranges from a scale of 0.5 to 5, 0.5 indicating green and 5 indicating yellow ground color.

Table 13. Effect of reflective mulches on starch breakdown of 'Rosy Glow' apples under permanent net at Paardekloof, Ceres (2019)

		Percentage starch breakdown	
Product			
	Control	51.63	ns
	Lumilys®	50.89	
	ColorIt	52.77	
Interaction (Position*Direction)			
	West Upper canopy	48.53	a
	West Lower canopy	49.27	a
	East Upper canopy	51.91	a
	East Lower canopy	57.35	b
Significance level			
	Product	*0.3209*	
	Position	*<.0001*	
	Direction	*0.0036*	
	*Product*Position*	*0.3251*	
	*Product*Direction*	*0.7205*	
	*Position*Direction*	***0.0244***	
	*Product*Position*Direction*	*0.1888*	

Table 14. Effect of reflective mulches on yield in ‘Fuji’ trees at Graymead, Vyeboom, South Africa (2019).

	Average yield per tree without draped net (kg)		Average yield efficiency per tree without draped net ($kg.cm^{-1}$)		Average yield per tree under draped net (kg)		Average yield efficiency per tree under draped net ($kg.cm^{-1}$)	
Product								
Control	31.01	ns	0.21	ns	21.59	ns	0.18	ns
Lumilys®	32.27		0.25		23.23		0.20	
ColorIt	32.29		0.23		20.04		0.16	
Position								
Upper canopy	29.54	ns	0.21	ns	22.48	ns	0.19	ns
Lower canopy	34.18		0.24		20.77		0.17	
Direction								
West	24.09	b	0.17	b	21.90	ns	0.18	ns
East	39.63	a	0.28	a	21.34		0.18	
Significance level								
Product	*0.9524*		*0.6395*		*0.3776*		*0.1790*	
Position	*0.2305*		*0.3447*		*0.3603*		*0.3346*	
Direction	***0.0002***		***0.0016***		*0.7623*		*0.6599*	
*Product*Position*	*0.9962*		*0.9831*		*0.9315*		*0.5008*	
*Product*Direction*	*0.8364*		*0.7611*		*0.8359*		*0.8825*	
*Position*Direction*	*0.7197*		*0.7393*		*0.2439*		*0.1899*	
*Product*Position*Direction*	*0.8064*		*0.7465*		*0.6217*		*0.7306*	

Table 15. Effect of reflective mulches on yield in 'Rosy Glow' apples at Paardekloof, Ceres, South Africa (2019).

	Average yield per tree without net (kg)		Average yield efficiency per tree without net ($kg.cm^{-1}$)		Average yield per tree under net (kg)		Average yield efficiency per tree under net ($kg.cm^{-1}$)	
Product								
Control	15.24	ns	0.31	a	26.53	ns	0.43	ns
Lumilys®	15.48		0.27	b	27.86		0.48	
ColorIt	15.37		0.31	a	29.15		0.47	
Position								
Upper canopy	15.11	b	0.29	ns	28.86	ns	0.48	ns
Lower canopy	15.62	a	0.30		26.83		0.44	
Direction								
West	15.26	ns	0.30	ns	29.70	a	0.50	a
East	15.47		0.29		25.98	b	0.43	b
Significance level								
Product	*0.5194*		***0.0036***		*0.2338*		*0.1893*	
Position	***0.0035***		*0.4063*		*0.1076*		*0.1269*	
Direction	*0.2392*		*0.4327*		***0.0040***		***0.0037***	
*Product*Position*	*0.4573*		*0.1467*		*0.5778*		***0.0266***	
*Product*Direction*	*0.2179*		*0.8857*		*0.3745*		*0.4389*	
*Position*Direction*	*0.0636*		*0.5481*		*0.2115*		*0.2673*	
*Product*Position*Direction*	*0.3519*		*0.9158*		*0.7553*		*0.9066*	

GENERAL DISCUSSION AND CONCLUSIONS

'Cripps' Pink' and older strains of 'Fuji' apples are notorious for poor and erratic red color development (Shafiq et al., 2011), especially in warmer climatic regions, such as the Elgin-Grabouw-Vyeboom-Villiersdorp (EGVV) region of South Africa. Many techniques exist that could potentially improve red color. The use of plant growth regulators (PGRs) is one such method that has shown potential to increase red color (Larrigaudiere et al., 1996; Shafiq et al., 2011; Stern et al., 2010), but also advance fruit maturity. Fruit harvested at post optimum maturity risk developing disorders such as diffuse internal browning during long term cold storage (Butler, 2015). The PGRs aminoethoxyvinylglycine (AVG), 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-methylcyclopropene (1-MCP) were evaluated on 'Cripps' Pink' in the EGVV to determine their effect on red color development, maturity and fruit quality. AVG is an ethylene inhibiting compound (Whale et al., 2008) that is utilized for harvest management by delaying fruit ripening when applied preharvest. 1-MCP can also be applied pre- or postharvest and inhibits the action of ethylene by blocking the ethylene receptors (Watkins, 2009), thereby halting the ripening process.

ACC (200 $\mu L \cdot L^{-1}$) applied two weeks before harvest (wbh) successfully increased the average blush percentage of 'Cripps' Pink' apples when combined and weighted over the first two picks during harvest. The same rate applied 1 wbh also increased percentage blush coverage, but the effect was less pronounced. AVG (ReTain®; 125 $mg \cdot L^{-1}$) delayed red color development when applied both one and 2 wbh. The combination of ACC 2 wbh with AVG 1 wbh, and vice versa, had a similar effect on percentage blush coverage as untreated control fruit, which was greater than AVG-treated fruit but less than ACC-treated fruit. AVG applied before or after ACC therefore partially reduced the efficacy of ACC to enhance red color development. The percentage of fruit qualifying to be marketed as Pink Lady™ weighted over two harvests mirrored the results observed in the percentage blush discussed before, but there was no effect of treatments on the percentage of 'Cripps' Pink' and third-class fruit. The increase in red color following ACC application is probably a response to upregulated ethylene synthesis stimulating fruit ripening and thus anthocyanin biosynthesis in the apple peel (Van de Poel and Van Der Straeten, 2014). The inverse is possibly true for the lack of red color development observed after AVG treatment, which competitively inhibits ACS and thus suppresses ethylene biosynthesis (Çetinbaş et al., 2012). For the same reasons, maturity was either advanced or delayed following ACC or AVG application, respectively. This is shown by the distribution in yield, where the greatest proportion of ACC-treated fruit was picked at the first harvest and the largest proportion AVG-treated fruit was picked at the third harvest. In addition, greater percentage starch breakdown and yellower ground color was noted in ACC-treated fruit compared to the untreated control at harvest, indicating advanced fruit maturity. In contrast, AVG-treated fruit displayed less starch breakdown and greener ground color than control fruit at harvest. Generally, the same observations were made after 12 weeks of RA storage at -0.5 °C and following seven days shelf-life at 20 °C regarding ground color of fruit. Interestingly, ground color of fruit treated with 1-MCP postharvest was similar to that of ACC-treated fruit (yellower than control) after storage, but when re-evaluated after seven days shelf-life, ground color was comparable to that of AVG treated fruit (greener than control). This inconsistency could therefore possibly be due to human error. ACC applied 2 wbh had the greatest overall effect on red color development in ‘Cripps’ Pink’ apples. However, maturity was also the most advanced. It was thus worthwhile to investigate the effect that different rates of ACC will have on both fruit color development and maturity when applied at 2 wbh.

Different concentrations of ACC, ranging from 100 $\mu L \cdot L^{-1}$ to 400 $\mu L \cdot L^{-1}$, were evaluated over two consecutive seasons on ‘Cripps’ Pink’ and one season on ‘Fuji Kiku’ (Brak) to establish the optimum ACC rate to induce red color development. The high rate of 400 $\mu L \cdot L^{-1}$ was included as it was estimated that a rate of 200 $\mu L \cdot L^{-1}$ will give optimal results in balancing color effects against early fruit ripening and for registration purposes the double rate is needed. A postharvest treatment of 1-MCP was also evaluated to establish whether this could counteract possible negative fruit ripening effects resulting from pre-harvest ACC treatment. An increase in internal ethylene concentration (IEC) in ‘Cripps’ Pink’ over time after ACC application in both seasons, was an indication that ACC was successfully absorbed by fruit and converted to ethylene. Since ethylene synthesis is autocatalytic (Ireland et al., 2014), the rise in IEC with time after application was expected. Red blush color of ‘Cripps’ Pink’ apples was successfully increased with increasing application rate of ACC for both seasons, which was similarly reflected in the percentage of fruit classified as Pink Lady™. This result is most probably due to the aforementioned increase in fruit IEC, since fruit ripening and therefore anthocyanin biosynthesis in apples are ethylene-mediated process (Saure, 1990). The same result regarding combined percentage blush coverage was not achieved in ‘Fuji’ apples at harvest, although a significant increase in the combined percentage of fruit with more than 50% red surface area over two harvests as ACC rate increased was noted, especially with the highest

ACC application rate (400 μL·L^{-1}). This may be due to some 'Fuji' strains experiencing a peak in red color quite a while before harvest, which then decreases towards harvest. If this is the case with 'Fuji Kiku' (Brak), it is uncertain whether application of ACC would improve red color in the apple peel, although according to Iglesias et al. (2012), a progressive increase in red color is seen in 'Fuji Kiku' apples before harvest. There was significant fruit drop in both cultivars with increasing ACC concentration, and therefore a general decrease in total yield per tree was observed as ACC rate increased. A shift in harvest maturity was noted in both cultivars, where a greater proportion of the total yield of ACC-treated plots was picked at the first harvest, gradually decreasing to third harvest. This is especially true for the higher rates of ACC (300 μl·L^{-1} and 400 μl·L^{-1}). High levels of starch breakdown for both 'Cripps' Pink' and 'Fuji' further emphasize the degree to which ACC advances maturity. It should be noted that fruit, especially those treated with higher ACC rates, were harvested post optimum maturity, with starch breakdown exceeding 30%, and ideally should have been harvested earlier compared to untreated control fruit. It is, however, questionable whether fruit harvested at optimum maturity would possess red color coverage similar to that of control fruit, since anthocyanin biosynthesis seems to be an indirectly stimulated by ACC application via stimulation of fruit ripening. This could be an interesting hypothesis for future studies. Postharvest treatment with 1-MCP, which inhibits ethylene activity (Tomala et al., 2020), maintained fruit maturity and storability of both cultivars, taking into consideration that ACC-treated fruit were already at post optimum maturity when placed into storage. Fruit treated with 1-MCP displayed greener ground color and greater flesh firmness than untreated fruit. Generally, 'Cripps' Pink' apples were more responsive to ACC than 'Fuji' apples. 'Fuji' apples produce low levels of ethylene (Doerflinger er al., 2019) and could thus have a less pronounced response to an increase in IEC due to upregulation of ethylene synthesis from ACC treatment. Unfortunately, the IEC of 'Fuji' was not determined in this trial to corroborate this. Future research on the cultivar specificity of ACC treatment would be beneficial to confirm the consistency of this observation. In order to confirm whether our finding of ACC at 200 μl·L^{-1} is effective in increasing red color development, with postharvest 1-MCP treatment to prevent advancing maturity of fruit, trials should be repeated whereby samples are harvested based on the time when each respective treatment is at optimum maturity (30% starch breakdown). In this way, sample color and maturity will be comparable among treatments and the untreated control and a more conclusive result should be obtained. Although, since ethylene stimulates ripening and indirectly anthocyanin synthesis, it remains questionable whether an improvement in red color coverage will be seen in lower and inner canopy fruit treated with ACC compared to the control.

Shade netting poses further problems to red color development, since anthocyanin synthesis is light mediated (Saure, 1990) and shade nets cause a decrease in fruit irradiance. To improve red color development without adversely affecting fruit maturity, two commercially available reflective mulches, viz. Lumilys® and ColorIt, were installed in ‘Fuji Brak’ (Kiku) and ‘Rosy Glow’ apple orchards with and without shade netting in the EGVV and Ceres areas, respectively, over one season. Both mulches performed equally throughout the trials. Photosynthetically active radiation (PAR) was increased by both reflective mulches under the net and in the open orchard. This is not only a valuable indication of increased light exposure to fruit. but may also have a positive effect on the photosynthetic efficiency of the orchard. This may help alleviate the especially taxing sink demands of fruit on tree assimilate availability during the growing season, resulting in overall higher quality fruit. The average percentage blush coverage and total Class one pack-out of ‘Fuji’ and ‘Rosy Glow’ apples were significantly increased by installation of either Lumilys® or ColorIt compared to the untreated control. Reflected light from the mulch has the greatest impact on lower canopy fruit, which are generally less exposed to sunlight and thus develop less red color. Mulch installation therefore resulted in a more uniform color development throughout the tree canopy, allowing lower and inner fruit, which are generally more mature than their upper and outer counterparts (ref), to be harvested at optimum maturity instead of being left on the tree until post optimum maturity, in order to obtain more red color. Neither mulch affected fruit size, and starch breakdown was either similar or less advanced than in control fruit. Although some varying results were obtained for fruit firmness in ‘Rosy Glow’ apples, all values were greater than the 6.5 $kg \cdot cm^{-2}$ minimum requirement for Pink Lady™. It can thus be concluded that in our trial fruit maturity was not negatively affected by either Lumilys® or ColorIt in ‘Fuji’ or ‘Rosy Glow’ apples. Cover crops were visually monitored and not affected by mulch in the orchard alley rows. Since the reflective fabrics were installed 10 cm above the soil and are of a woven nature, water percolation through both mulches could occur freely. This allows soil ecology to remain uninterrupted by mulch installation. Although both mulches are quite expensive, the increase in Class one pack-out from increased red color coverage as well as the possibility to reuse the mulches within a season on multiple cultivars and over multiple seasons should justify the cost.

Conclusion

1-Aminocyclopropane-1-carboxylic acid (ACC) successfully increased red color development of ‘Cripps’ Pink’ apples when applied two weeks before harvest. Less pronounced results were obtained on ‘Fuji’ apples and this should be further investigated. However, fruit drop and advanced fruit maturity were problematic, especially at high rates of ACC. The 200 $\mu L \cdot L^{-1}$ ACC application two wbh followed by postharvest 1-MCP treatment was promising, but further research is needed to quantify the advantages of this treatment. Both Lumilys® and ColorIt reflective mulches resulted in a significant increase in red color development of especially problematic lower canopy fruit in the open orchard and under shade net, without negatively affecting fruit maturity of ‘Fuji’ and ‘Rosy Glow’ apples. Although input costs are high, it should be justified by the potential increase in profits from Class one pack-out and the fact that mulches can be re-used within the season as well as over multiple seasons. For both the PGR and reflective mulch approach an economical study should be done. Although, based on our study, reflective mulch installation four to five weeks before harvest would be recommended for improving red color development in apples, without negatively affecting fruit maturity.

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